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TAASTRUP 03. Jun 1999

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## NOVEL MANNANASES

The present invention relates to microbial mannanases, more specifically to microbial enzymes exhibiting mannanase activity  
5 as their major enzymatic activity in the neutral and alkaline pH ranges; to a method of producing such enzymes; and to methods for using such enzymes in the paper and pulp, textile, cleaning and cellulose fiber processing industries.

## 10 BACKGROUND OF THE INVENTION

Mannan containing polysaccharides are a major component of the hemicellulose fraction in woods and endosperm in many leguminous seeds and in some mature seeds of non-leguminous plants. Essentially unsubstituted linear beta-1,4-mannan is  
15 found in some non-leguminous plants. Unsubstituted beta-1,4-mannan which is present e.g. in ivory nuts resembles cellulose in the conformation of the individual polysaccharide chains, and is water-insoluble. In leguminous seeds, water-soluble galactomannan is the main storage carbohydrate comprising up to  
20 20% of the total dry weight. Galactomannans have a linear beta-1,4-mannan backbone substituted with single alpha-1,6-galactose, optionally substituted with acetyl groups. Mannans are also found in several monocotyledonous plants and are the most abundant polysaccharides in the cell wall material in palm  
25 kernel meal. Glucomannans are linear polysaccharides with a backbone of beta-1,4-linked mannose and glucose alternating in a more or less regular manner, the backbone optionally being substituted with galactose and/or acetyl groups. Mannans, galactomannans, glucomannans and galactoglucomannans (i.e.  
30 glucomannan backbones with branched galactose) contribute to more than 50% of the softwood hemicellulose. Moreover, the cellulose of many red algae contains a significant amount of

mannose.

Mannanases have been identified in several *Bacillus* organisms. For example, Talbot et al., Appl. Environ. Microbiol., Vol.56, No. 11, pp. 3505-3510 (1990) describes a  
5 beta-mannanase derived from *Bacillus stearothermophilus* in dimer form having molecular weight of 162 kDa and an optimum pH of 5.5-7.5. Mendoza et al., World J. Microbiol. Biotech., Vol. 10, No. 5, pp. 551-555 (1994) describes a beta-mannanase derived from *Bacillus subtilis* having a molecular weight of 38 kDa, an  
10 optimum activity at pH 5.0 and 55°C and a pI of 4.8. JP-03047076 discloses a beta-mannanase derived from *Bacillus* sp., having a molecular weight of 37±3 kDa measured by gel filtration, an optimum pH of 8-10 and a pI of 5.3-5.4. JP-63056289 describes the production of an alkaline, thermostable beta-mannanase which  
15 hydrolyses beta-1,4-D-mannopyranoside bonds of e.g. mannans and produces manno-oligosaccharides. JP-63036774 relates to the *Bacillus* microorganism FERM P-8856 which produces beta-mannanase and beta-mannosidase at an alkaline pH. JP-08051975 discloses alkaline beta-mannanases from alkalophilic *Bacillus* sp. AM-001.  
20 A purified mannanase from *Bacillus amyloliquefaciens* useful in the bleaching of pulp and paper and a method of preparation thereof is disclosed in WO 97/11164. WO 91/18974 describes a hemicellulase such as a glucanase, xylanase or mannanase active at an extreme pH and temperature. WO 94/25576 discloses an  
25 enzyme from *Aspergillus aculeatus*, CBS 101.43, exhibiting mannanase activity which may be useful for degradation or modification of plant or algae cell wall material. WO 93/24622 discloses a mannanase isolated from *Trichoderma reseei* useful for bleaching lignocellulosic pulps.  
30 WO 95/35362 discloses cleaning compositions containing plant cell wall degrading enzymes having pectinase and/or hemicellulase and optionally cellulase activity for the removal

of stains of vegetable origin and further discloses an alkaline mannanase from the strain C11SB.G17.

It is an object of the present invention to provide a novel and efficient enzyme exhibiting mannanase activity also in the  
5 alkaline pH range, e.g. when applied in cleaning compositions or different industrial processes.

#### SUMMARY OF THE INVENTION

The inventors have now found a novel enzyme having  
10 substantial mannanase activity, i.e. an enzyme exhibiting mannanase activity which may be obtained from a bacterial strain of the genus *Bacillus*, more specifically of the strain *Bacillus halodurans*, and have succeeded in identifying a DNA sequence encoding such enzyme. The DNA sequence and the deduced amino  
15 acid sequence are listed in the sequence listing as SEQ ID No. 1 and 2, respectively. It is believed that the novel enzyme will be classified according to the Enzyme Nomenclature in the Enzyme Class EC 3.2.1.78.

In a first aspect, the present invention relates to a  
20 mannanase which is i) a polypeptide produced by *Bacillus halodurans*, ii) a polypeptide comprising an amino acid sequence as shown in positions 33-331 of SEQ ID NO:2, or iii) an analogue of the polypeptide defined in i) or ii) which is at least 65% homologous with said polypeptide, is derived from said  
25 polypeptide by substitution, deletion or addition of one or several amino acids, or is immunologically reactive with a polyclonal antibody raised against said polypeptide in purified form.

Within one aspect, the present invention provides an iso-  
30 lated polynucleotide molecule selected from the group consisting of (a) polynucleotide molecules encoding a polypeptide having mannanase activity and comprising a sequence of nucleotides as



shown in SEQ ID NO: 1 from nucleotide 138 to nucleotide 1034;  
(b) species homologs of (a); (c) polynucleotide molecules that  
encode a polypeptide having mannanase activity that is at least  
65% identical to the amino acid sequence of SEQ ID NO: 2 from  
5 amino acid residue 33 to amino acid residue 331; (d) molecules  
complementary to (a), (b) or (c); and (e) degenerate nucleotide  
sequences of (a), (b), (c) or (d).

The plasmid pBXM5 comprising the polynucleotide molecule  
(the DNA sequence) encoding a mannanase of the present invention  
10 has been transformed into a strain of the *Escherichia coli* which  
was deposited by the inventors according to the Budapest Treaty  
on the International Recognition of the Deposit of  
Microorganisms for the Purposes of Patent Procedure at the  
Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH,  
15 Mascheroder Weg 1b, D-38124 Braunschweig, Federal Republic of  
Germany, on 9 October 1998 under the deposition number DSM  
12441.

Within another aspect of the invention there is provided an  
expression vector comprising the following operably linked  
20 elements: a transcription promoter; a DNA segment selected from  
the group consisting of (a) polynucleotide molecules encoding a  
polypeptide having mannanase activity and comprising a sequence  
of nucleotides as shown in SEQ ID NO: 1 from nucleotide 138 to  
nucleotide 1034; (b) species homologs of (a); (c) polynucleotide  
25 molecules that encode a polypeptide having mannanase activity  
that is at least 65% identical to the amino acid sequence of SEQ  
ID NO: 2 from amino acid residue 33 to amino acid residue 331;  
and (d) degenerate nucleotide sequences of (a), (b), or (c); and  
a transcription terminator.

30 Within yet another aspect of the present invention there is  
provided a cultured cell into which has been introduced an

expression vector as disclosed above, wherein said cell expresses the polypeptide encoded by the DNA segment.

A further aspect of the present invention provides an isolated polypeptide having mannanase activity selected from the group consisting of (a) polypeptide molecules comprising a sequence of amino acid residues as shown in SEQ ID NO:2 from amino acid residue 33 to amino acid residue 331; (b) species homologs of (a).

Within another aspect of the present invention there is provided a composition comprising a purified polypeptide according to the invention in combination with other polypeptides.

Within another aspect of the present invention there are provided methods for producing a polypeptide according to the invention comprising culturing a cell into which has been introduced an expression vector as disclosed above, whereby said cell expresses a polypeptide encoded by the DNA segment and recovering the polypeptide.

The novel enzyme of the present invention is useful for the treatment of cellulosic material, especially cellulose-containing fiber, yarn, woven or non-woven fabric, treatment of mechanical paper-making pulps, kraft pulps or recycled waste paper, and for retting of fibres. The treatment can be carried out during the processing of cellulosic material into a material ready for manufacture of paper or of garment or fabric, the latter e.g. in the desizing or scouring step; or during industrial or household laundering of such fabric or garment.

Accordingly, in further aspects the present invention relates to a cleaning or detergent composition comprising an enzyme having substantial mannanase activity; and to use of the enzyme of the invention for the treatment, eg cleaning, of cellulose-containing fibers, yarn, woven or non-woven fabric, as well as synthetic or partly synthetic fabric.

The enzyme of the invention is very effective for use in an enzymatic scouring process and/or desizing (removal of mannan size) in the preparation of cellulosic material e.g. for proper response in subsequent dyeing operations. The enzyme is also  
5 useful for removal of mannan containing print paste. Further, detergent compositions comprising the novel enzyme are capable of removing or bleaching certain soils or stains present on laundry, especially soils and spots resulting from mannan containing food, plants, and the like. Further, treatment with  
10 cleaning or detergent compositions comprising the novel enzyme can prevent binding of certain soils to the cellulosic material.

Accordingly, the present invention also relates to cleaning compositions, including laundry, dishwashing, hard surface cleaner, personal cleansing and oral/dental compositions, com-  
15 prising a mannanase. Further, the present invention relates to such cleaning compositions comprising a mannanase and an enzyme selected from cellulases, amylases, pectin degrading enzymes and/or xyloglucanases, such compositions providing superior cleaning performance, i.e. superior stain removal, dingy clean-  
20 ing and whiteness maintenance.

#### DEFINITIONS

Prior to discussing this invention in further detail, the following terms will first be defined.

25 The term "ortholog" (or "species homolog") denotes a polypeptide or protein obtained from one species that has homology to an analogous polypeptide or protein from a different species.

The term "paralog" denotes a polypeptide or protein obtained  
30 from a given species that has homology to a distinct polypeptide or protein from that same species.

The term "expression vector" denotes a DNA molecule, linear or circular, that comprises a segment encoding a polypeptide of interest operably linked to additional segments that provide for its transcription. Such additional segments may include promoter and terminator sequences, and may optionally include one or more origins of replication, one or more selectable markers, an enhancer, a polyadenylation signal, and the like. Expression vectors are generally derived from plasmid or viral DNA, or may contain elements of both. The expression vector of the invention may be any expression vector that is conveniently subjected to recombinant DNA procedures, and the choice of vector will often depend on the host cell into which the vector is to be introduced. Thus, the vector may be an autonomously replicating vector, i.e. a vector which exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication, e.g. a plasmid. Alternatively, the vector may be one which, when introduced into a host cell, is integrated into the host cell genome and replicated together with the chromosome(s) into which it has been integrated.

The term "recombinant expressed" or "recombinantly expressed" used herein in connection with expression of a polypeptide or protein is defined according to the standard definition in the art. Recombinantly expression of a protein is generally performed by using an expression vector as described immediately above.

The term "isolated", when applied to a polynucleotide molecule, denotes that the polynucleotide has been removed from its natural genetic milieu and is thus free of other extraneous or unwanted coding sequences, and is in a form suitable for use within genetically engineered protein production systems. Such isolated molecules are those that are separated from their natural environment and include cDNA and genomic clones. Iso-

lated DNA molecules of the present invention are free of other genes with which they are ordinarily associated, but may include naturally occurring 5' and 3' untranslated regions such as promoters and terminators. The identification of associated  
5 regions will be evident to one of ordinary skill in the art (see for example, Dynan and Tijan, Nature 316:774-78, 1985). The term "an isolated polynucleotide" may alternatively be termed "a cloned polynucleotide".

When applied to a protein/polypeptide, the term "isolated" indicates that the protein is found in a condition other than its native environment. In a preferred form, the isolated protein is substantially free of other proteins, particularly other homologous proteins (i.e. "homologous impurities" (see below)). It is preferred to provide the protein in a greater than 40%  
15 pure form, more preferably greater than 60% pure form.

Even more preferably it is preferred to provide the protein in a highly purified form, i.e., greater than 80% pure, more preferably greater than 95% pure, and even more preferably greater than 99% pure, as determined by SDS-PAGE.

20 The term "isolated protein/polypeptide may alternatively be termed "purified protein/polypeptide".

The term "homologous impurities" means any impurity (e.g. another polypeptide than the polypeptide of the invention) which originate from the homologous cell where the polypeptide of the  
25 invention is originally obtained from.

The term "obtained from" as used herein in connection with a specific microbial source, means that the polynucleotide and/or polypeptide produced by the specific source, or by a cell in which a gene from the source have been inserted.

30 The term "operably linked", when referring to DNA segments, denotes that the segments are arranged so that they function in concert for their intended purposes, e.g. transcription initi-

ates in the promoter and proceeds through the coding segment to the terminator

The term "polynucleotide" denotes a single- or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases read from the 5' to the 3' end. Polynucleotides include RNA and DNA, and may be isolated from natural sources, synthesized *in vitro*, or prepared from a combination of natural and synthetic molecules.

The term "complements of polynucleotide molecules" denotes polynucleotide molecules having a complementary base sequence and reverse orientation as compared to a reference sequence. For example, the sequence 5' ATGCACGGG 3' is complementary to 5' CCCGTGCAT 3'.

The term "degenerate nucleotide sequence" denotes a sequence of nucleotides that includes one or more degenerate codons (as compared to a reference polynucleotide molecule that encodes a polypeptide). Degenerate codons contain different triplets of nucleotides, but encode the same amino acid residue (i.e., GAU and GAC triplets each encode Asp).

The term "promoter" denotes a portion of a gene containing DNA sequences that provide for the binding of RNA polymerase and initiation of transcription. Promoter sequences are commonly, but not always, found in the 5' non-coding regions of genes.

The term "secretory signal sequence" denotes a DNA sequence that encodes a polypeptide (a "secretory peptide") that, as a component of a larger polypeptide, directs the larger polypeptide through a secretory pathway of a cell in which it is synthesized. The larger peptide is commonly cleaved to remove the secretory peptide during transit through the secretory pathway.

The term "mannanase" or "galactomannanase" denotes a mannanase enzyme defined according to the art as officially being named mannan endo-1,4-beta-mannosidase and having the alterna-

tive names beta-mannanase and endo-1,4-mannanase and catalysing the reaction: random hydrolyses of 1,4-beta-D-mannosidic linkages in mannans, galactomannans, glucomannans, and galactoglucomannans.

5

#### DETAILED DESCRIPTION OF THE INVENTION

HOW TO USE A SEQUENCE OF THE INVENTION TO GET OTHER RELATED SEQUENCES: The disclosed sequence information herein relating to a polynucleotide sequence encoding a mannanase of the invention can be used as a tool to identify other homologous mannanases. For instance, polymerase chain reaction (PCR) can be used to amplify sequences encoding other homologous mannanases from a variety of microbial sources, in particular of different *Bacillus* species.

15

#### POLYNUCLEOTIDES:

Within preferred embodiments of the invention an isolated polynucleotide of the invention will hybridize to similar sized regions of SEQ ID No. 1, or a sequence complementary thereto, under at least medium stringency conditions.

In particular polynucleotides of the invention will hybridize to a denatured double-stranded DNA probe comprising either the full sequence shown in positions 42-1051 of SEQ ID NO:1 or the subsequence shown in positions 42-1034 of SEQ ID NO:1 or the subsequence shown in positions 138-1034 of SEQ ID NO:1 or any probe comprising a subsequence of SEQ ID NO:1 having a length of at least about 100 base pairs under at least medium stringency conditions, but preferably at high stringency conditions as described in detail below. Suitable experimental conditions for determining hybridization at medium, or high stringency between a nucleotide probe and a homologous DNA or RNA sequence involves presoaking of the filter containing the

DNA fragments or RNA to hybridize in 5 x SSC (Sodium chloride/Sodium citrate, Sambrook et al. 1989) for 10 min, and prehybridization of the filter in a solution of 5 x SSC, 5 x Denhardt's solution (Sambrook et al. 1989), 0.5 % SDS and 100  
5 µg/ml of denatured sonicated salmon sperm DNA (Sambrook et al. 1989), followed by hybridization in the same solution containing a concentration of 10ng/ml of a random-primed (Feinberg, A. P. and Vogelstein, B. (1983) Anal. Biochem. 132:6-13), <sup>32</sup>P-dCTP-labeled (specific activity higher than 1 x 10<sup>9</sup> cpm/µg ) probe  
10 for 12 hours at ca. 45°C. The filter is then washed twice for 30 minutes in 2 x SSC, 0.5 % SDS at least 60°C (medium stringency), still more preferably at least 65°C (medium/high stringency), even more preferably at least 70°C (high stringency), and even more preferably at least 75°C (very high stringency).

15 Molecules to which the oligonucleotide probe hybridizes under these conditions are detected using a x-ray film.

As previously noted, the isolated polynucleotides of the present invention include DNA and RNA. Methods for isolating DNA and RNA are well known in the art. DNA and RNA encoding  
20 genes of interest can be cloned in Gene Banks or DNA libraries by means of methods known in the art.

Polynucleotides encoding polypeptides having mannanase activity of the invention are then identified and isolated by, for example, hybridization or PCR.

25 The present invention further provides counterpart polypeptides and polynucleotides from different bacterial strains (orthologs or paralogs). Of particular interest are mannanase polypeptides from gram-positive alkalophilic strains, including species of *Bacillus*.

30 Species homologues of a polypeptide with mannanase activity of the invention can be cloned using information and compositions provided by the present invention in combination



with conventional cloning techniques. For example, a DNA sequence of the present invention can be cloned using chromosomal DNA obtained from a cell type that expresses the protein. Suitable sources of DNA can be identified by probing Northern blots with probes designed from the sequences disclosed herein. A library is then prepared from chromosomal DNA of a positive cell line. A DNA sequence of the invention encoding an polypeptide having mannanase activity can then be isolated by a variety of methods, such as by probing with probes designed from the sequences disclosed in the present specification and claims or with one or more sets of degenerate probes based on the disclosed sequences. A DNA sequence of the invention can also be cloned using the polymerase chain reaction, or PCR (Mullis, U.S. Patent 4,683,202), using primers designed from the sequences disclosed herein. Within an additional method, the DNA library can be used to transform or transfect host cells, and expression of the DNA of interest can be detected with an antibody (monoclonal or polyclonal) raised against the mannanase cloned from *B.halodurans*, expressed and purified as described in Materials and Methods and Example 1, or by an activity test relating to a polypeptide having mannanase activity.

The mannanase encoding part of the DNA sequence cloned into plasmid pBXM5 present in *Escherichia coli* 12441 and/or an analogue DNA sequence of the invention may be cloned from a strain of the bacterial species *Bacillus halodurans*, or another or related organism as described herein.

Alternatively, the analogous sequence may be constructed on the basis of the DNA sequence obtainable from the plasmid present in *Escherichia coli* DSM 12441 (which is believed to be identical to the attached SEQ ID NO:1), e.g be a sub-sequence thereof, and/or by introduction of nucleotide substitutions which do not give rise to another amino acid sequence of the

mannanase encoded by the DNA sequence, but which corresponds to the codon usage of the host organism intended for production of the enzyme, or by introduction of nucleotide substitutions which may give rise to a different amino acid sequence (*i.e.* a variant  
5 of the mannan degrading enzyme of the invention).

#### POLYPEPTIDES:

The sequence of amino acids nos. 33-331 of SEQ ID NO: 2 is believed to be a mature mannanase sequence. Since the object of  
10 the present invention is to obtain a polypeptide which exhibits mannanase activity, the present invention relates to any mannanase enzyme comprising the sequence of amino acids nos. 33-331 of SEQ ID NO: 2, ie a catalytical domain, optionally operably linked, either N-terminally or C-terminally, to one or  
15 two other domains of a different functionality.

The present invention also provides mannanase polypeptides that are substantially homologous to the polypeptide of SEQ ID NO:2 and species homologs (paralogs or orthologs) thereof. The term "substantially homologous" is used herein to denote  
20 polypeptides having 65%, preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, and even more preferably at least 90%, sequence identity to the sequence shown in amino acids nos. 33-331 of SEQ ID NO:2 or its orthologs or paralogs. Such  
25 polypeptides will more preferably be at least 95% identical, and most preferably 98% or more identical to the sequence shown in amino acids nos. 1-331 of SEQ ID NO:2 or its orthologs or paralogs. Percent sequence identity is determined by conventional methods, by means of computer programs known in the  
30 art such as GAP provided in the GCG program package (Program Manual for the Wisconsin Package, Version 8, August 1994, Genetics Computer Group, 575 Science Drive, Madison, Wisconsin,

USA 53711) as disclosed in Needleman, S.B. and Wunsch, C.D., (1970), Journal of Molecular Biology, 48, 443-453, which is hereby incorporated by reference in its entirety. GAP is used with the following settings for polypeptide sequence comparison:  
5 GAP creation penalty of 3.0 and GAP extension penalty of 0.1.

Sequence identity of polynucleotide molecules is determined by similar methods using GAP with the following settings for DNA sequence comparison: GAP creation penalty of 5.0 and GAP extension penalty of 0.3.

The enzyme preparation of the invention is preferably derived from a microorganism, preferably from a bacterium, an archaea or a fungus, especially from a bacterium such as a bacterium belonging to *Bacillus*, preferably to an alkalophilic *Bacillus* strain which may be selected from the group consisting of the species *Bacillus halodurans* and highly related *Bacillus* species in which all species preferably are at least 93%, more preferably at least 95%, even more preferably at least 98%, homologous to *Bacillus halodurans* based on aligned 16S rDNA sequences.

10        These species can be found and identified based on phylogenetic relationships identified from aligned 16S rDNA sequences from RDP (Ribosomal Database Project) (Bonne L. Maidak, Neils Larson, Michael J. McCaughey, Ross Overbeek, Gary J. Olsen, Karl Fogel, James Blandy, and Carl R. Woese, Nucleic Acids  
15        Reasearch, 1994, Vol. 22, No17, p. 3485-3487, The Ribosomal Database Project). The alignment was based on secondary structure. Calculation of sequence similarities were established using the "Full matrix calculation" with default settings of the neighbor joining method integrated in the ARB program pack-  
20        age (Oliver Strunk and Wolfgang Ludwig, Technical University of Munich, Germany). Based on this alignment it is contemplated that the *Bacillus* strain used as donor organism for the gene

encoding for the mannanase of the present invention is a strain belonging to the species *Bacillus halodurans*. Accordingly, the present invention relates to family 5 mannanases derived from the highly related *Bacillus* species in which all species show  
 5 more than 93% homology to *Bacillus halodurans* based on aligned 16S sequences. These *Bacillus* species include: *Sporolactobacillus laevis*, *Bacillus agaradhaerens* and *Marinococcus halophilus*. See Figure 1: Phylogenic tree generated from ARP program relating closest species to *Bacillus halodurans*.

10 **Table II:** 16S ribosomal RNA homology index for selected *Bacillus* species

	SplLaev3	BaiSpec6	BaiSpe11	MaoHalo2	NN
SplLaev3		90.98%	87.96%	85.94%	91.32%
BaiSpec6			91.63%	87.96%	99.46%
15 BaiSpe11				89.04%	92.04%
MaoHalo2					88.17%
NN					

SplLaev3 = *Sporolactobacillus laevis*, D16287

20 BaiSpec6 = *B. halodurans*, X76442

BaiSpe11 = *B. agaradhaerens*, X76445

MaoHalo2 = *Marinococcus halophilus*, X62171

NN = donor organism of the invention (*B. halodurans*)

25 Substantially homologous proteins and polypeptides are characterized as having one or more amino acid substitutions, deletions or additions. These changes are preferably of a minor nature, that is conservative amino acid substitutions (see Table 2) and other substitutions that do not significantly affect the  
 30 folding or activity of the protein or polypeptide; small deletions, typically of one to about 30 amino acids; and small amino- or carboxyl-terminal extensions, such as an amino-

terminal methionine residue, a small linker peptide of up to about 20-25 residues, or a small extension that facilitates purification (an affinity tag), such as a poly-histidine tract, protein A (Nilsson et al., EMBO J. 4:1075, 1985; Nilsson et al.,  
 5 Methods Enzymol. 198:3, 1991. See, in general Ford et al.,  
Protein Expression and Purification 2: 95-107, 1991, which is incorporated herein by reference. DNAs encoding affinity tags are available from commercial suppliers (e.g., Pharmacia Biotech, Piscataway, NJ; New England Biolabs, Beverly, MA).

10 However, even though the changes described above preferably are of a minor nature, such changes may also be of a larger nature such as fusion of larger polypeptides of up to 300 amino acids or more both as amino- or carboxyl-terminal extensions to a Mannanase polypeptide of the invention.

15

Table 1

Conservative amino acid substitutions

20	Basic:	arginine
		lysine
		histidine
	Acidic:	glutamic acid
		aspartic acid
	Polar:	glutamine
		asparagine
25	Hydrophobic:	leucine
		isoleucine
		valine
30	Aromatic:	phenylalanine
		tryptophan
		tyrosine
	Small:	glycine

alanine  
serine  
threonine  
methionine

5

In addition to the 20 standard amino acids, non-standard amino acids (such as 4-hydroxyproline, 6-N-methyl lysine, 2-aminoisobutyric acid, isovaline and α-methyl serine) may be substituted for amino acid residues of a polypeptide according to the invention. A limited number of non-conservative amino acids, amino acids that are not encoded by the genetic code, and unnatural amino acids may be substituted for amino acid residues. "Unnatural amino acids" have been modified after protein synthesis, and/or have a chemical structure in their side chain(s) different from that of the standard amino acids. Unnatural amino acids can be chemically synthesized, or preferably, are commercially available, and include pipecolic acid, thiazolidine carboxylic acid, dehydroproline, 3- and 4-methylproline, and 3,3-dimethylproline.

Essential amino acids in the mannanase polypeptides of the present invention can be identified according to procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, Science 244: 1081-1085, 1989). In the latter technique, single alanine mutations are introduced at every residue in the molecule, and the resultant mutant molecules are tested for biological activity (i.e. mannanase activity) to identify amino acid residues that are critical to the activity of the molecule. See also, Hilton et al., J. Biol. Chem. 271:4699-4708, 1996. The active site of the enzyme or other biological interaction can also be determined by physical analysis of structure, as determined by such techniques as nuclear magnetic resonance, crystallography, electron dif-

fraction or photoaffinity labeling, in conjunction with mutation of putative contact site amino acids. See, for example, de Vos et al., Science 255:306-312, 1992; Smith et al., J. Mol. Biol. 224:899-904, 1992; Wlodaver et al., FEBS Lett. 309:59-64, 1992.

5 The identities of essential amino acids can also be inferred from analysis of homologies with polypeptides which are related to a polypeptide according to the invention.

Multiple amino acid substitutions can be made and tested using known methods of mutagenesis, recombination and/or shuffling followed by a relevant screening procedure, such as those disclosed by Reidhaar-Olson and Sauer (Science 241:53-57, 1988), Bowie and Sauer (Proc. Natl. Acad. Sci. USA 86:2152-2156, 1989), WO95/17413, or WO 95/22625. Briefly, these authors disclose methods for simultaneously randomizing two or more positions in  
15 a polypeptide, or recombination/shuffling of different mutations (WO95/17413, WO95/22625), followed by selecting for functional a polypeptide, and then sequencing the mutagenized polypeptides to determine the spectrum of allowable substitutions at each position. Other methods that can be used include phage display  
20 (e.g., Lowman et al., Biochem. 30:10832-10837, 1991; Ladner et al., U.S. Patent No. 5,223,409; Huse, WIPO Publication WO 92/06204) and region-directed mutagenesis (Derbyshire et al., Gene 46:145, 1986; Ner et al., DNA 7:127, 1988).

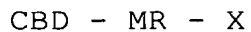
Mutagenesis/shuffling methods as disclosed above can be combined with high-throughput, automated screening methods to  
25 detect activity of cloned, mutagenized polypeptides in host cells. Mutagenized DNA molecules that encode active polypeptides can be recovered from the host cells and rapidly sequenced using modern equipment. These methods allow the rapid determination of  
30 the importance of individual amino acid residues in a polypeptide of interest, and can be applied to polypeptides of unknown structure.

Using the methods discussed above, one of ordinary skill in the art can identify and/or prepare a variety of polypeptides that are substantially homologous to residues 33 to 331 of SEQ ID NO: 2 and retain the mannanase activity of the wild-type  
5 protein.

The mannanase enzyme of the invention may, in addition to the enzyme core comprising the catalytically domain, also comprise a cellulose binding domain (CBD), the cellulose binding domain and enzyme core (the catalytically active domain) of the enzyme being operably linked. The cellulose binding domain (CBD) may exist as an integral part of the encoded enzyme, or a CBD from another origin may be introduced into the mannan degrading enzyme thus creating an enzyme hybrid. In this context, the term "cellulose-binding domain" is intended to be understood as defined by Peter Tomme et al. "Cellulose-Binding Domains: Classification and Properties" in "Enzymatic Degradation of Insoluble Carbohydrates", John N. Saddler and Michael H. Penner (Eds.), ACS Symposium Series, No. 618, 1996. This definition classifies more than 120 cellulose-binding domains into 10 families (I-X), and demonstrates that CBDs are found in various enzymes such as cellulases, xylanases, mannanases, arabinofuranosidases, acetyl esterases and chitinases. CBDs have also been found in algae, e.g. the red alga *Porphyra purpurea* as a non-hydrolytic polysaccharide-binding protein, see Tomme et al., *op.cit.* However, most of the CBDs are from cellulases and xylanases, CBDs are found at the N and C termini of proteins or are internal. Enzyme hybrids are known in the art, see e.g. WO 90/00609 and WO 95/16782, and may be prepared by transforming into a host cell a DNA construct comprising at least a fragment of DNA encoding the cellulose-binding domain ligated, with or without a linker, to a DNA sequence encoding the mannan degrading enzyme and growing the host cell to ex-



press the fused gene. Enzyme hybrids may be described by the following formula:



wherein CBD is the N-terminal or the C-terminal region of an amino acid sequence corresponding to at least the cellulose-binding domain; MR is the middle region (the linker), and may be  
5 a bond, or a short linking group preferably of from about 2 to about 100 carbon atoms, more preferably of from 2 to 40 carbon atoms; or is preferably from about 2 to to about 100 amino acids, more preferably of from 2 to 40 amino acids; and X is an N-terminal or C-terminal region of the mannanase of the  
10 invention.

Preferably, the mannanase enzyme of the present invention has its maximum catalytic activity at a pH of at least 8, more preferably of at least 8.5, more preferably of at least 9, more preferably of at least 9.5, more preferably of at least 10, even  
15 more preferably of at least 10.5, especially of at least 11; and preferably the maximum activity of the enzyme is obtained at a temperature of at least 50°C, more preferably of at least 55°C.

Preferably, the cleaning composition of the present invention provides, eg when used for treating fabric during a washing  
20 cycle of a machine washing process, a washing solution having a pH typically between about 8 and about 10.5. Typically, such a washing solution is used at temperatures between about 20°C and about 95°C, preferably between about 20°C and about 60°C, preferably between about 20°C and about 50°C.

25

#### PROTEIN PRODUCTION:

The proteins and polypeptides of the present invention, including full-length proteins, fragments thereof and fusion proteins, can be produced in genetically engineered host cells  
30 according to conventional techniques. Suitable host cells are

those cell types that can be transformed or transfected with exogenous DNA and grown in culture, and include bacteria, fungal cells, and cultured higher eukaryotic cells. Bacterial cells, particularly cultured cells of gram-positive organisms, are preferred. Gram-positive cells from the genus of *Bacillus* are especially preferred, such as from the group consisting of *Bacillus subtilis*, *Bacillus lentus*, *Bacillus brevis*, *Bacillus stearothermophilus*, *Bacillus alkalophilus*, *Bacillus amyloliquefaciens*, *Bacillus coagulans*, *Bacillus circulans*, *Bacillus lautus*, *Bacillus thuringiensis*, *Bacillus licheniformis*, and *Bacillus sp.*, in particular *Bacillus halodurans*.

Techniques for manipulating cloned DNA molecules and introducing exogenous DNA into a variety of host cells are disclosed by Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989; Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley and Sons, Inc., NY, 1987; and "Bacillus subtilis and Other Gram-Positive Bacteria", Sonensheim et al., 1993, American Society for Microbiology, Washington D.C., which are incorporated herein by reference.

In general, a DNA sequence encoding a mannanase of the present invention is operably linked to other genetic elements required for its expression, generally including a transcription promoter and terminator within an expression vector. The vector will also commonly contain one or more selectable markers and one or more origins of replication, although those skilled in the art will recognize that within certain systems selectable markers may be provided on separate vectors, and replication of the exogenous DNA may be provided by integration into the host cell genome. Selection of promoters, terminators, selectable markers, vectors and other elements is a matter of routine design within the level of ordinary skill in the art. Many such

elements are described in the literature and are available through commercial suppliers.

To direct a polypeptide into the secretory pathway of a host cell, a secretory signal sequence (also known as a leader sequence, prepro sequence or pre sequence) is provided in the expression vector. The secretory signal sequence may be that of the polypeptide, or may be derived from another secreted protein or synthesized *de novo*. Numerous suitable secretory signal sequences are known in the art and reference is made to "Bacillus subtilis and Other Gram-Positive Bacteria", Sonensheim et al., 1993, American Society for Microbiology, Washington D.C.; and Cutting, S. M. (eds.) "Molecular Biological Methods for Bacillus", John Wiley and Sons, 1990, for further description of suitable secretory signal sequences especially for secretion in a Bacillus host cell. The secretory signal sequence is joined to the DNA sequence in the correct reading frame. Secretory signal sequences are commonly positioned 5' to the DNA sequence encoding the polypeptide of interest, although certain signal sequences may be positioned elsewhere in the DNA sequence of interest (see, e.g., Welch et al., U.S. Patent No. 5,037,743; Holland et al., U.S. Patent No. 5,143,830).

Transformed or transfected host cells are cultured according to conventional procedures in a culture medium containing nutrients and other components required for the growth of the chosen host cells. A variety of suitable media, including defined media and complex media, are known in the art and generally include a carbon source, a nitrogen source, essential amino acids, vitamins and minerals. Media may also contain such components as growth factors or serum, as required. The growth medium will generally select for cells containing the exogenously added DNA by, for example, drug selection or deficiency in an essential

nutrient which is complemented by the selectable marker carried on the expression vector or co-transfected into the host cell.

#### PROTEIN ISOLATION:

5        When the expressed recombinant polypeptide is secreted the polypeptide may be purified from the growth media. Preferably the expression host cells are removed from the media before purification of the polypeptide (e.g. by centrifugation).

10        When the expressed recombinant polypeptide is not secreted from the host cell, the host cell are preferably disrupted and the polypeptide released into an aqueous "extract" which is the first stage of such purification techniques. Preferably the expression host cells are collected from the media before the cell disruption (e.g. by centrifugation).

15        The cell disruption may be performed by conventional techniques such as by lysozyme digestion or by forcing the cells through high pressure. See (Robert K. Scobes, Protein Purification, Second edition, Springer-Verlag) for further description of such cell disruption techniques.

20        Whether or not the expressed recombinant polypeptides (or chimeric polypeptides) is secreted or not it can be purified using fractionation and/or conventional purification methods and media.

25        Ammonium sulfate precipitation and acid or chaotrope extraction may be used for fractionation of samples. Exemplary purification steps may include hydroxyapatite, size exclusion, FPLC and reverse-phase high performance liquid chromatography. Suitable anion exchange media include derivatized dextrans, agarose, cellulose, polyacrylamide, specialty silicas, and the like. PEI, 30 DEAE, QAE and Q derivatives are preferred, with DEAE Fast-Flow Sepharose (Pharmacia, Piscataway, NJ) being particularly preferred. Exemplary chromatographic media include those media

derivatized with phenyl, butyl, or octyl groups, such as Phenyl-Sepharose FF (Pharmacia), Toyopearl butyl 650 (Toso Haas, Montgomeryville, PA), Octyl-Sepharose (Pharmacia) and the like; or polyacrylic resins, such as Amberchrom CG 71 (Toso Haas) and the like. Suitable solid supports include glass beads, silica-based resins, cellulosic resins, agarose beads, cross-linked agarose beads, polystyrene beads, cross-linked polyacrylamide resins and the like that are insoluble under the conditions in which they are to be used. These supports may be modified with reactive groups that allow attachment of proteins by amino groups, carboxyl groups, sulfhydryl groups, hydroxyl groups and/or carbohydrate moieties. Examples of coupling chemistries include cyanogen bromide activation, N-hydroxysuccinimide activation, epoxide activation, sulfhydryl activation, hydrazide activation, and carboxyl and amino derivatives for carbodiimide coupling chemistries. These and other solid media are well known and widely used in the art, and are available from commercial suppliers.

Selection of a particular method is a matter of routine design and is determined in part by the properties of the chosen support. See, for example, Affinity Chromatography: Principles & Methods, Pharmacia LKB Biotechnology, Uppsala, Sweden, 1988.

Polypeptides of the invention or fragments thereof may also be prepared through chemical synthesis. Polypeptides of the invention may be monomers or multimers; glycosylated or non-glycosylated; pegylated or non-pegylated; and may or may not include an initial methionine amino acid residue.

Based on the sequence information disclosed herein a full length DNA sequence encoding a mannanase of the invention and comprising the DNA sequence shown in SEQ ID No 1, at least the DNA sequence from position 138 to position 1034, or, alterna-

tively, the DNA sequence from position 42 to position 1034 or from position 42 to position 1051, may be cloned.

Cloning is performed by standard procedures known in the art such as by,

- 5 ■ preparing a genomic library from a *Bacillus* strain, especially the strain *B. halodurans*;
- plating such a library on suitable substrate plates;
- identifying a clone comprising a polynucleotide sequence of the invention by standard hybridization techniques using a
- 10 probe based on SEQ ID No 1; or by
- identifying a clone from said *Bacillus halodurans* genomic library by an Inverse PCR strategy using primers based on sequence information from SEQ ID No 1. Reference is made to
- 15 M.J. MCPherson et al. ("PCR A practical approach" Information Press Ltd, Oxford England) for further details relating to Inverse PCR.

Based on the sequence information disclosed herein (SEQ ID No 1, SEQ ID No 2) is it routine work for a person skilled in the art to isolate homologous polynucleotide sequences encoding homologous mannanase of the invention by a similar

20 strategy using genomic libraries from related microbial organisms, in particular from genomic libraries from other strains of the genus *Bacillus* such as alkalophilic species of *Bacillus halodurans*.

25 Alternatively, the DNA encoding the mannan or galactomannan-degrading enzyme of the invention may, in accordance with well-known procedures, conveniently be cloned from a suitable source, such as any of the above mentioned organisms, by use of synthetic oligonucleotide probes prepared on the basis of the

30 DNA sequence obtainable from the plasmid present in *Escherichia coli* DSM 12441.

Accordingly, the polynucleotide molecule of the invention may be isolated from *Escherichia coli*, DSM 12441, in which the plasmid obtained by cloning such as described above is deposited. Also, the present invention relates to an isolated substantially pure biological culture of the strain *Escherichia coli*, DSM 12441.

#### TRANSGENIC PLANTS

The present invention also relates to a transgenic plant, plant part or plant cell which has been transformed with a DNA  
5 sequence encoding the mannanase of the invention so as to express and produce this enzyme in recoverable quantities. The enzyme may be recovered from the plant or plant part. Alternatively, the plant or plant part containing the recombinant enzyme may be used as such.

10 The transgenic plant can be dicotyledonous or monocotyledonous, for short a dicot or a monocot. Examples of monocot plants are grasses, such as meadow grass (blue grass, Poa), forage grass such as festuca, lolium, temperate grass, such as Agrostis, and cereals, e.g. wheat, oats, rye, barley,  
15 rice, sorghum and maize (corn).

Examples of dicot plants are tobacco, legumes, such as lupins, potato, sugar beet, pea, bean and soybean, and cruciferous (family Brassicaceae), such as cauliflower, oil seed rape and the closely related model organism Arabidopsis  
20 thaliana.

Examples of plant parts are stem, callus, leaves, root, fruits, seeds, and tubers. In the present context, also specific plant tissues, such as chloroplast, apoplast, mitochondria, vacuole, peroxisomes and cytoplasm are considered to be a plant  
25 part. Furthermore, any plant cell, whatever the tissue origin, is considered to be a plant part.

Also included within the scope of the invention are the progeny of such plants, plant parts and plant cells.

The transgenic plant or plant cell expressing the enzyme of the invention may be constructed in accordance with methods known in the art. In short the plant or plant cell is constructed by incorporating one or more expression constructs encoding the enzyme of the invention into the plant host genome and propagating the resulting modified plant or plant cell into a transgenic plant or plant cell.

10 Conveniently, the expression construct is a DNA construct which comprises a gene encoding the enzyme of the invention in operable association with appropriate regulatory sequences required for expression of the gene in the plant or plant part of choice. Furthermore, the expression construct may comprise a  
15 selectable marker useful for identifying host cells into which the expression construct has been integrated and DNA sequences necessary for introduction of the construct into the plant in question (the latter depends on the DNA introduction method to be used).

20 The choice of regulatory sequences, such as promoter and terminator sequences and optionally signal or transit sequences is determined, eg on the basis of when, where and how the enzyme is desired to be expressed. For instance, the expression of the gene encoding the enzyme of the invention may be constitutive or  
25 inducible, or may be developmental, stage or tissue specific, and the gene product may be targeted to a specific tissue or plant part such as seeds or leaves. Regulatory sequences are eg described by Tague et al, Plant, Phys., 86, 506, 1988.

For constitutive expression the 35S-CaMV promoter may be  
30 used (Franck et al., 1980. Cell 21: 285-294). Organ-specific promoters may eg be a promoter from storage sink tissues such as seeds, potato tubers, and fruits (Edwards & Coruzzi, 1990. Annu.



Rev. Genet. 24: 275-303), or from metabolic sink tissues such as meristems (Ito et al., 1994. Plant Mol. Biol. 24: 863-878), a seed specific promoter such as the glutelin, prolamin, globulin or albumin promoter from rice (Wu et al., Plant and Cell Physiology Vol. 39, No. 8 pp. 885-889 (1998)), a *Vicia faba* promoter from the legumin B4 and the unknown seed protein gene from *Vicia faba* described by Conrad U. et al, Journal of Plant Physiology Vol. 152, No. 6 pp. 708-711 (1998), a promoter from a seed oil body protein (Chen et al., Plant and cell physiology vol. 39, No. 9 pp. 935-941 (1998), the storage protein napA promoter from *Brassica napus*, or any other seed specific promoter known in the art, eg as described in WO 91/14772. Furthermore, the promoter may be a leaf specific promoter such as the rbcS promoter from rice or tomato (Kyoizuka et al., Plant Physiology Vol. 102, No. 3 pp. 991-1000 (1993), the chlorella virus adenine methyltransferase gene promoter (Mitra, A. and Higgins, DW, Plant Molecular Biology Vol. 26, No. 1 pp. 85-93 (1994), or the aldP gene promoter from rice (Kagaya et al., Molecular and General Genetics Vol. 248, No. 6 pp. 668-674 (1995), or a wound inducible promoter such as the potato pin2 promoter (Xu et al, Plant Molecular Biology Vol. 22, No. 4 pp. 573-588 (1993)).

A promoter enhancer element may be used to achieve higher expression of the enzyme in the plant. For instance, the promoter enhancer element may be an intron which is placed between the promoter and the nucleotide sequence encoding the enzyme. For instance, Xu et al. *op cit* disclose the use of the first intron of the rice actin 1 gene to enhance expression.

The selectable marker gene and any other parts of the expression construct may be chosen from those available in the art.

The DNA construct is incorporated into the plant genome according to conventional techniques known in the art, including

*Agrobacterium*-mediated transformation, virus-mediated transformation, micro injection, particle bombardment, biolistic transformation, and electroporation (Gasser et al, Science, 244, 1293; Potrykus, Bio/Techn. 8, 535, 1990; Shimamoto et al, Nature, 338, 274, 1989).

Presently, *Agrobacterium tumefaciens* mediated gene transfer is the method of choice for generating transgenic dicots (for review Hooykas & Schilperoort, 1992. Plant Mol. Biol. 19: 15-38), however it can also be used for transforming monocots, although other transformation methods are generally preferred for these plants. Presently, the method of choice for generating transgenic monocots is particle bombardment (microscopic gold or tungsten particles coated with the transforming DNA) of embryonic calli or developing embryos (Christou, 1992. Plant J. 2: 275-281; Shimamoto, 1994. Curr. Opin. Biotechnol. 5: 158-162; Vasil et al., 1992. Bio/Technology 10: 667-674). An alternative method for transformation of monocots is based on protoplast transformation as described by Omirulleh S, et al., Plant Molecular biology Vol. 21, No. 3 pp. 415-428 (1993).

Following transformation, the transformants having incorporated the expression construct are selected and regenerated into whole plants according to methods well-known in the art.

#### ENZYME PREPARATION

In the present context, the term "enzyme preparation" is intended to mean either a conventional enzymatic fermentation product, possibly isolated and purified, from a single species of a microorganism, such preparation usually comprising a number of different enzymatic activities; or a mixture of monocomponent enzymes, preferably enzymes derived from bacterial or fungal

species by using conventional recombinant techniques, which enzymes have been fermented and possibly isolated and purified separately and which may originate from different species, preferably fungal or bacterial species; or the fermentation  
5 product of a microorganism which acts as a host cell for expression of a recombinant mannanase, but which microorganism simultaneously produces other enzymes, e.g. pectin degrading enzymes, proteases, or cellulases, being naturally occurring fermentation products of the microorganism, i.e. the enzyme  
10 complex conventionally produced by the corresponding naturally occurring microorganism.

The mannanase preparation of the invention may further comprise one or more enzymes selected from the group consisting of proteases, cellulases (endo- $\beta$ -1,4-glucanases),  $\beta$ -glucanases  
15 (endo- $\beta$ -1,3(4)-glucanases), lipases, cutinases, peroxidases, laccases, amylases, glucoamylases, pectinases, reductases, oxidases, phenoloxidases, ligninases, pullulanases, hemicellulases, pectate lyases, xyloglucanases, xylanases, pectin acetyl esterases, rhamnogalacturonan acetyl esterases,  
20 polygalacturonases, rhamnogalacturonases, pectin lyases, pectin methylesterases, cellobiohydrolases, transglutaminases; or mixtures thereof. In a preferred embodiment, one or more or all enzymes in the preparation is produced by using recombinant techniques, i.e. the enzyme(s) is/are mono-component enzyme(s)  
25 which is/are mixed with the other enzyme(s) to form an enzyme preparation with the desired enzyme blend.

In another aspect, the present invention also relates to a method of producing the enzyme preparation of the invention, the method comprising culturing a microorganism, eg a wild-type  
30 strain, capable of producing the mannanase under conditions permitting the production of the enzyme, and recovering the enzyme from the culture. Culturing may be carried out using

conventional fermentation techniques, e.g. culturing in shake flasks or fermentors with agitation to ensure sufficient aeration on a growth medium inducing production of the mannanase enzyme. The growth medium may contain a conventional N-source  
5 such as peptone, yeast extract or casamino acids, a reduced amount of a conventional C-source such as dextrose or sucrose, and an inducer such as guar gum or locust bean gum. The recovery may be carried out using conventional techniques, e.g. separation of bio-mass and supernatant by centrifugation or  
10 filtration, recovery of the supernatant or disruption of cells if the enzyme of interest is intracellular, perhaps followed by further purification as described in EP 0 406 314 or by crystallization as described in WO 97/15660.

#### 15 IMMUNOLOGICAL CROSS-REACTIVITY

Monospecific polyclonal antibodies to be used in determining immunological cross-reactivity may be prepared by use of a purified mannanase enzyme. More specifically, antiserum against the mannanase of the invention may be raised by  
20 immunizing rabbits (or other rodents) according to the procedure described by N. Axelsen et al. in: A Manual of Quantitative Immuno-electrophoresis, Blackwell Scientific Publications, 1973, Chapter 23, or A. Johnstone and R. Thorpe, Immunochemistry in Practice, Blackwell Scientific Publications, 1982 (more speci-  
25 fically p. 27-31). Purified immunoglobulins may be obtained from the antisera, for example by salt precipitation  $((\text{NH}_4)_2 \text{SO}_4)$ , followed by dialysis and ion exchange chromatography, e.g. on DEAE-Sephadex. Immunochemical characterization of proteins may be done either by Ouchterlony double-diffusion analysis (O.  
30 Ouchterlony in: Handbook of Experimental Immunology (D.M. Weir, Ed.), Blackwell Scientific Publications, 1967, pp. 655-706), by crossed immuno-electrophoresis (N. Axelsen et al., supra, Chap-

ters 3 and 4), or by rocket immunoelectrophoresis (N. Axelsen et al., Chapter 2).

Examples of useful bacteria producing the enzyme or the  
5 enzyme preparation of the invention are Gram positive bacteria, preferably from the *Bacillus/Lactobacillus* subdivision, preferably a strain from the genus *Bacillus*, more preferably a strain of *Bacillus halodurans*.

In yet another aspect, the present invention relates to an  
10 isolated mannanase having the properties described above and which is free from homologous impurities, and is produced using conventional recombinant techniques.

#### **Use in the detergent industry**

15 In further aspects, the present invention relates to a detergent composition comprising the mannanase or mannanase preparation of the invention, to a process for machine treatment of fabrics comprising treating fabric during a washing cycle of a machine washing process with a washing solution containing the  
20 mannanase or mannanase preparation of the invention, and to cleaning compositions, including laundry, dishwashing, hard surface cleaner, personal cleansing and oral/dental compositions, comprising a mannanase and optionally another enzyme selected among cellulases, amylases, pectin degrading enzymes  
25 and xyloglucanases and providing superior cleaning performance, i.e. superior stain removal, dingy cleaning and whiteness maintenance.

Without being bound to this theory, it is believed that the mannanase of the present invention is capable of effectively  
30 degrading or hydrolysing any soiling or spots containing galactomannans and, accordingly, of cleaning laundry comprising such soilings or spots.

The cleaning compositions of the invention must contain at least one additional detergent component. The precise nature of these additional components, and levels of incorporation thereof will depend on the physical form of the composition, and the  
5 nature of the cleaning operation for which it is to be used.

The cleaning compositions of the present invention preferably further comprise a detergent ingredient selected from a selected surfactant, another enzyme, a builder and/or a bleach system.

10 The cleaning compositions according to the invention can be liquid, paste, gels, bars, tablets, spray, foam, powder or granular. Granular compositions can also be in "compact" form and the liquid compositions can also be in a "concentrated" form.

15 The compositions of the invention may for example, be formulated as hand and machine dishwashing compositions, hand and machine laundry detergent compositions including laundry additive compositions and compositions suitable for use in the soaking and/or pretreatment of stained fabrics, rinse added  
20 fabric softener compositions, and compositions for use in general household hard surface cleaning operations. Compositions containing such carbohydrases can also be formulated as sanitization products, contact lens cleansers and health and beauty care products such as oral/dental care and personal cleaning  
25 compositions.

When formulated as compositions for use in manual dishwashing methods the compositions of the invention preferably contain a surfactant and preferably other detergent compounds selected from organic polymeric compounds, suds enhancing agents, group  
30 II metal ions, solvents, hydrotropes and additional enzymes.

When formulated as compositions suitable for use in a laundry machine washing method, the compositions of the inven-

tion preferably contain both a surfactant and a builder compound and additionally one or more detergent components preferably selected from organic polymeric compounds, bleaching agents, additional enzymes, suds suppressors, dispersants, lime-soap  
5 dispersants, soil suspension and anti-redeposition agents and corrosion inhibitors. Laundry compositions can also contain softening agents, as additional detergent components. Such compositions containing carbohydrase can provide fabric cleaning, stain removal, whiteness maintenance, softening, colour  
10 appearance, dye transfer inhibition and sanitization when formulated as laundry detergent compositions.

The compositions of the invention can also be used as detergent additive products in solid or liquid form. Such additive products are intended to supplement or boost the performance of conventional detergent compositions and can be added at  
15 any stage of the cleaning process.

If needed the density of the laundry detergent compositions herein ranges from 400 to 1200 g/litre, preferably 500 to 950 g/litre of composition measured at 20°C.

20 The "compact" form of the compositions herein is best reflected by density and, in terms of composition, by the amount of inorganic filler salt; inorganic filler salts are conventional ingredients of detergent compositions in powder form; in conventional detergent compositions, the filler salts are present in substantial amounts, typically 17-35% by weight of the  
25 total composition. In the compact compositions, the filler salt is present in amounts not exceeding 15% of the total composition, preferably not exceeding 10%, most preferably not exceeding 5% by weight of the composition. The inorganic filler salts,  
30 such as meant in the present compositions are selected from the alkali and alkaline-earth-metal salts of sulphates and chlorides. A preferred filler salt is sodium sulphate.

Liquid detergent compositions according to the present invention can also be in a "concentrated form", in such case, the liquid detergent compositions according the present invention will contain a lower amount of water, compared to conventional liquid detergents. Typically the water content of the concentrated liquid detergent is preferably less than 40%, more preferably less than 30%, most preferably less than 20% by weight of the detergent composition.

10 Cleaning compositions  
Surfactant system

The cleaning or detergent compositions according to the present invention comprise a surfactant system, wherein the surfactant can be selected from nonionic and/or anionic and/or cationic and/or ampholytic and/or zwitterionic and/or semi-polar surfactants.

The surfactant is typically present at a level from 0.1% to 60% by weight. The surfactant is preferably formulated to be compatible with enzyme hybrid and enzyme components present in the composition. In liquid or gel compositions the surfactant is most preferably formulated in such a way that it promotes, or at least does not degrade, the stability of any enzyme hybrid or enzyme in these compositions.

Suitable systems for use according to the present invention comprise as a surfactant one or more of the nonionic and/or anionic surfactants described herein.

Polyethylene, polypropylene, and polybutylene oxide condensates of alkyl phenols are suitable for use as the nonionic surfactant of the surfactant systems of the present invention, with the polyethylene oxide condensates being preferred. These compounds include the condensation products of alkyl phenols having an alkyl group containing from about 6 to



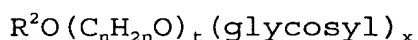
about 14 carbon atoms, preferably from about 8 to about 14 carbon atoms, in either a straight chain or branched-chain configuration with the alkylene oxide. In a preferred embodiment, the ethylene oxide is present in an amount equal to from about 2  
5 to about 25 moles, more preferably from about 3 to about 15 moles, of ethylene oxide per mole of alkyl phenol. Commercially available nonionic surfactants of this type include Igepal<sup>TM</sup> CO-630, marketed by the GAF Corporation; and Triton<sup>TM</sup> X-45, X-114, X-100 and X-102, all marketed by the Rohm & Haas Company. These  
10 surfactants are commonly referred to as alkylphenol alkoxylates (e.g., alkyl phenol ethoxylates).

The condensation products of primary and secondary aliphatic alcohols with about 1 to about 25 moles of ethylene oxide are suitable for use as the nonionic surfactant of the  
15 nonionic surfactant systems of the present invention. The alkyl chain of the aliphatic alcohol can either be straight or branched, primary or secondary, and generally contains from about 8 to about 22 carbon atoms. Preferred are the condensation products of alcohols having an alkyl group containing from about  
20 8 to about 20 carbon atoms, more preferably from about 10 to about 18 carbon atoms, with from about 2 to about 10 moles of ethylene oxide per mole of alcohol. About 2 to about 7 moles of ethylene oxide and most preferably from 2 to 5 moles of ethylene oxide per mole of alcohol are present in said condensation pro-  
25 ducts. Examples of commercially available nonionic surfactants of this type include Tergitol<sup>TM</sup> 15-S-9 (The condensation product of C<sub>11</sub>-C<sub>15</sub> linear alcohol with 9 moles ethylene oxide), Tergitol<sup>TM</sup> 24-L-6 NMW (the condensation product of C<sub>12</sub>-C<sub>14</sub> primary alcohol with 6 moles ethylene oxide with a narrow molecular weight  
30 distribution), both marketed by Union Carbide Corporation; Neodol<sup>TM</sup> 45-9 (the condensation product of C<sub>14</sub>-C<sub>15</sub> linear alcohol with 9 moles of ethylene oxide), Neodol<sup>TM</sup> 23-3 (the condensation

product of C<sub>12</sub>-C<sub>13</sub> linear alcohol with 3.0 moles of ethylene oxide), Neodol™ 45-7 (the condensation product of C<sub>14</sub>-C<sub>15</sub> linear alcohol with 7 moles of ethylene oxide), Neodol™ 45-5 (the condensation product of C<sub>14</sub>-C<sub>15</sub> linear alcohol with 5 moles of ethylene oxide) marketed by Shell Chemical Company, Kyro™ EOB (the condensation product of C<sub>13</sub>-C<sub>15</sub> alcohol with 9 moles ethylene oxide), marketed by The Procter & Gamble Company, and Genapol LA 050 (the condensation product of C<sub>12</sub>-C<sub>14</sub> alcohol with 5 moles of ethylene oxide) marketed by Hoechst. Preferred range of HLB in these products is from 8-11 and most preferred from 8-10.

Also useful as the nonionic surfactant of the surfactant systems of the present invention are alkylpolysaccharides disclosed in US 4,565,647, having a hydrophobic group containing from about 6 to about 30 carbon atoms, preferably from about 10 to about 16 carbon atoms and a polysaccharide, e.g. a polyglycoside, hydrophilic group containing from about 1.3 to about 10, preferably from about 1.3 to about 3, most preferably from about 1.3 to about 2.7 saccharide units. Any reducing saccharide containing 5 or 6 carbon atoms can be used, e.g., glucose, galactose and galactosyl moieties can be substituted for the glucosyl moieties (optionally the hydrophobic group is attached at the 2-, 3-, 4-, etc. positions thus giving a glucose or galactose as opposed to a glucoside or galactoside). The intersaccharide bonds can be, e.g., between the one position of the additional saccharide units and the 2-, 3-, 4-, and/or 6-positions on the preceding saccharide units.

The preferred alkylpolyglycosides have the formula



wherein R<sup>2</sup> is selected from the group consisting of alkyl, alkylphenyl, hydroxyalkyl, hydroxyalkylphenyl, and mixtures thereof in which the alkyl groups contain from about 10 to about

18, preferably from about 12 to about 14, carbon atoms; n is 2 or 3, preferably 2; t is from 0 to about 10, preferably 0; and x is from about 1.3 to about 10, preferably from about 1.3 to about 3, most preferably from about 1.3 to about 2.7. The  
5 glycosyl is preferably derived from glucose. To prepare these compounds, the alcohol or alkylpolyethoxy alcohol is formed first and then reacted with glucose, or a source of glucose, to form the glucoside (attachment at the 1-position). The additional glycosyl units can then be attached between their 1-  
10 position and the preceding glycosyl units 2-, 3-, 4-, and/or 6-position, preferably predominantly the 2-position.

The condensation products of ethylene oxide with a hydrophobic base formed by the condensation of propylene oxide with propylene glycol are also suitable for use as the  
15 additional nonionic surfactant systems of the present invention. The hydrophobic portion of these compounds will preferably have a molecular weight from about 1500 to about 1800 and will exhibit water insolubility. The addition of polyoxyethylene moieties to this hydrophobic portion tends to increase the water  
20 solubility of the molecule as a whole, and the liquid character of the product is retained up to the point where the polyoxyethylene content is about 50% of the total weight of the condensation product, which corresponds to condensation with up to about 40 moles of ethylene oxide. Examples of compounds of  
25 this type include certain of the commercially available Pluronic<sup>TM</sup> surfactants, marketed by BASF.

Also suitable for use as the nonionic surfactant of the nonionic surfactant system of the present invention, are the condensation products of ethylene oxide with the product  
30 resulting from the reaction of propylene oxide and ethylenediamine. The hydrophobic moiety of these products consists of the reaction product of ethylenediamine and excess

propylene oxide, and generally has a molecular weight of from about 2500 to about 3000. This hydrophobic moiety is condensed with ethylene oxide to the extent that the condensation product contains from about 40% to about 80% by weight of

5 polyoxyethylene and has a molecular weight of from about 5,000 to about 11,000. Examples of this type of nonionic surfactant include certain of the commercially available Tetronic™ compounds, marketed by BASF.

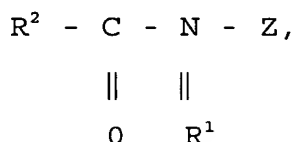
Preferred for use as the nonionic surfactant of the

10 surfactant systems of the present invention are polyethylene oxide condensates of alkyl phenols, condensation products of primary and secondary aliphatic alcohols with from about 1 to about 25 moles of ethyleneoxide, alkylpolysaccharides, and mixtures hereof. Most preferred are C<sub>8</sub>-C<sub>14</sub> alkyl phenol

15 ethoxylates having from 3 to 15 ethoxy groups and C<sub>8</sub>-C<sub>18</sub> alcohol ethoxylates (preferably C<sub>10</sub> avg.) having from 2 to 10 ethoxy groups, and mixtures thereof.

Highly preferred nonionic surfactants are polyhydroxy fatty acid amide surfactants of the formula

20



wherein R<sup>1</sup> is H, or R<sup>1</sup> is C<sub>1-4</sub> hydrocarbyl, 2-hydroxyethyl, 2-

25 hydroxypropyl or a mixture thereof, R<sup>2</sup> is C<sub>5-31</sub> hydrocarbyl, and Z is a polyhydroxyhydrocarbyl having a linear hydrocarbyl chain with at least 3 hydroxyls directly connected to the chain, or an alkoxylated derivative thereof. Preferably, R<sup>1</sup> is methyl, R<sup>2</sup> is straight C<sub>11-15</sub> alkyl or C<sub>16-18</sub> alkyl or alkenyl chain such as

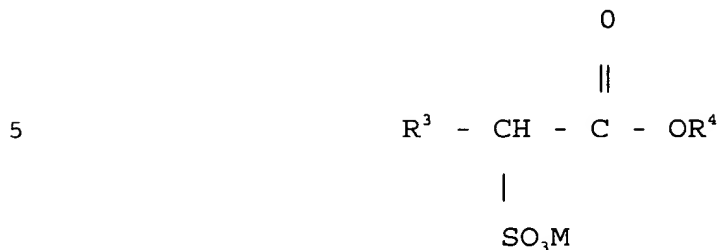
30 coconut alkyl or mixtures thereof, and Z is derived from a reducing sugar such as glucose, fructose, maltose or lactose, in a reductive amination reaction.

Highly preferred anionic surfactants include alkyl alkoxyated sulfate surfactants. Examples hereof are water soluble salts or acids of the formula  $RO(A)_mSO_3M$  wherein R is an unsubstituted  $C_{10}-C_{24}$  alkyl or hydroxyalkyl group having a  $C_{10}-C_{24}$  alkyl component, preferably a  $C_{12}-C_{20}$  alkyl or hydro-xyalkyl, more preferably  $C_{12}-C_{18}$  alkyl or hydroxyalkyl, A is an ethoxy or propoxy unit, m is greater than zero, typically between about 0.5 and about 6, more preferably between about 0.5 and about 3, and M is H or a cation which can be, for example, a metal cation (e.g., sodium, potassium, lithium, calcium, magnesium, etc.), ammonium or substituted-ammonium cation. Alkyl ethoxyated sulfates as well as alkyl propoxyated sulfates are contemplated herein. Specific examples of substituted ammonium cations include methyl-, dimethyl, trimethyl-ammonium cations and quaternary ammonium cations such as tetramethyl-ammonium and dimethyl piperdinium cations and those derived from alkylamines such as ethylamine, diethylamine, triethylamine, mixtures thereof, and the like. Exemplary surfactants are  $C_{12}-C_{18}$  alkyl polyethoxylate (1.0) sulfate ( $C_{12}-C_{18}E(1.0)M$ ),  $C_{12}-C_{18}$  alkyl polyethoxylate (2.25) sulfate ( $C_{12}-C_{18}(2.25)M$ ), and  $C_{12}-C_{18}$  alkyl polyethoxylate (3.0) sulfate ( $C_{12}-C_{18}E(3.0)M$ ), and  $C_{12}-C_{18}$  alkyl polyethoxylate (4.0) sulfate ( $C_{12}-C_{18}E(4.0)M$ ), wherein M is conveniently selected from sodium and potassium.

Suitable anionic surfactants to be used are alkyl ester sulfonate surfactants including linear esters of  $C_8-C_{20}$  carboxylic acids (i.e., fatty acids) which are sulfonated with gaseous  $SO_3$  according to "The Journal of the American Oil Chemists Society", 52 (1975), pp. 323-329. Suitable starting materials would include natural fatty substances as derived from tallow, palm oil, etc.

The preferred alkyl ester sulfonate surfactant, especially for laundry applications, comprise alkyl ester sulfonate

surfactants of the structural formula:



wherein  $R^3$  is a  $C_8$ - $C_{20}$  hydrocarbyl, preferably an alkyl, or combination thereof,  $R^4$  is a  $C_1$ - $C_6$  hydrocarbyl, preferably an alkyl, or combination thereof, and M is a cation which forms a water soluble salt with the alkyl ester sulfonate. Suitable salt-forming cations include metals such as sodium, potassium, and lithium, and substituted or unsubstituted ammonium cations, such as monoethanolamine, diethanolamine, and triethanolamine.

15 Preferably,  $R^3$  is  $C_{10}$ - $C_{16}$  alkyl, and  $R^4$  is methyl, ethyl or isopropyl. Especially preferred are the methyl ester sulfonates wherein  $R^3$  is  $C_{10}$ - $C_{16}$  alkyl.

Other suitable anionic surfactants include the alkyl sulfate surfactants which are water soluble salts or acids of the formula  $ROSO_3M$  wherein R preferably is a  $C_{10}$ - $C_{24}$  hydrocarbyl, preferably an alkyl or hydroxyalkyl having a  $C_{10}$ - $C_{20}$  alkyl component, more preferably a  $C_{12}$ - $C_{18}$  alkyl or hydroxyalkyl, and M is H or a cation, e.g., an alkali metal cation (e.g. sodium, potassium, lithium), or ammonium or substituted ammonium (e.g. methyl-, dimethyl-, and trimethyl ammonium cations and quaternary ammonium cations such as tetramethyl-ammonium and dimethyl piperdinium cations and quaternary ammonium cations derived from alkylamines such as ethylamine, diethylamine, triethylamine, and mixtures thereof, and the like). Typically,

30 alkyl chains of  $C_{12}$ - $C_{16}$  are preferred for lower wash temperatures (e.g. below about  $50^\circ\text{C}$ ) and  $C_{16}$ - $C_{18}$  alkyl chains are preferred for higher wash temperatures (e.g. above about  $50^\circ\text{C}$ ).

Other anionic surfactants useful for deterative purposes can also be included in the laundry detergent compositions of the present invention. These can include salts (including, for example, sodium, potassium, ammonium, and substituted ammonium salts such as mono- di- and triethanolamine salts) of soap, C<sub>8</sub>-C<sub>22</sub> primary or secondary alkanesulfonates, C<sub>8</sub>-C<sub>24</sub> olefinsulfonates, sulfonated polycarboxylic acids prepared by sulfonation of the pyrolyzed product of alkaline earth metal citrates, e.g., as described in British patent specification No. 1,082,179, C<sub>8</sub>-C<sub>24</sub> alkylpolyglycolethersulfates (containing up to 10 moles of ethylene oxide); alkyl glycerol sulfonates, fatty acyl glycerol sulfonates, fatty oleyl glycerol sulfates, alkyl phenol ethylene oxide ether sulfates, paraffin sulfonates, alkyl phosphates, isethionates such as the acyl isethionates, N-acyl taurates, alkyl succinamates and sulfosuccinates, monoesters of sulfosuccinates (especially saturated and unsaturated C<sub>12</sub>-C<sub>18</sub> monoesters) and diesters of sulfosuccinates (especially saturated and unsaturated C<sub>6</sub>-C<sub>12</sub> diesters), acyl sarcosinates, sulfates of alkylpolysaccharides such as the sulfates of alkylpolyglucoside (the nonionic nonsulfated compounds being described below), branched primary alkyl sulfates, and alkyl polyethoxy carboxylates such as those of the formula  $RO(CH_2CH_2O)_k-CH_2COO-M^+$  wherein R is a C<sub>8</sub>-C<sub>22</sub> alkyl, k is an integer from 1 to 10, and M is a soluble salt forming cation. Resin acids and hydrogenated resin acids are also suitable, such as rosin, hydrogenated rosin, and resin acids and hydrogenated resin acids present in or derived from tall oil.

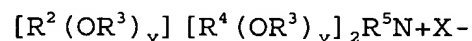
Alkylbenzene sulfonates are highly preferred. Especially preferred are linear (straight-chain) alkyl benzene sulfonates (LAS) wherein the alkyl group preferably contains from 10 to 18 carbon atoms.

Further examples are described in "Surface Active Agents and Detergents" (Vol. I and II by Schwartz, Perry and Berch). A variety of such surfactants are also generally disclosed in US 3,929,678, (Column 23, line 58 through Column 29, line 23,  
5 herein incorporated by reference).

When included therein, the laundry detergent compositions of the present invention typically comprise from about 1% to about 40%, preferably from about 3% to about 20% by weight of such anionic surfactants.

10 The cleaning or laundry detergent compositions of the present invention may also contain cationic, ampholytic, zwitterionic, and semi-polar surfactants, as well as the nonionic and/or anionic surfactants other than those already described herein.

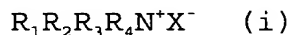
15 Cationic deterative surfactants suitable for use in the laundry detergent compositions of the present invention are those having one long-chain hydrocarbyl group. Examples of such cationic surfactants include the ammonium surfactants such as alkyltrimethylammonium halogenides, and those surfactants having  
20 the formula:





wherein  $R^2$  is an alkyl or alkyl benzyl group having from about 8 to about 18 carbon atoms in the alkyl chain, each  $R^3$  is selected from the group consisting of  $-CH_2CH_2-$ ,  $-CH_2CH(CH_3)-$ ,  $-CH_2CH(CH_2OH)-$ ,  $-CH_2CH_2CH_2-$ , and mixtures thereof; each  $R^4$  is  
 5 selected from the group consisting of  $C_1-C_4$  alkyl,  $C_1-C_4$  hydroxyalkyl, benzyl ring structures formed by joining the two  $R^4$  groups,  $-CH_2CHOHCHOHCOR^6CHOHCH_2OH$ , wherein  $R^6$  is any hexose or hexose polymer having a molecular weight less than about 1000, and hydrogen when  $y$  is not 0;  $R^5$  is the same as  $R^4$  or is an alkyl  
 10 chain, wherein the total number of carbon atoms or  $R^2$  plus  $R^5$  is not more than about 18; each  $y$  is from 0 to about 10, and the sum of the  $y$  values is from 0 to about 15; and  $X$  is any compatible anion.

Highly preferred cationic surfactants are the water  
 15 soluble quaternary ammonium compounds useful in the present composition having the formula:



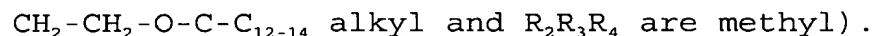
wherein  $R_1$  is  $C_8-C_{16}$  alkyl, each of  $R_2$ ,  $R_3$  and  $R_4$  is independently  
 20  $C_1-C_4$  alkyl,  $C_1-C_4$  hydroxy alkyl, benzyl, and  $-(C_2H_4)_xH$  where  $x$  has a value from 2 to 5, and  $X$  is an anion. Not more than one of  $R_2$ ,  $R_3$  or  $R_4$  should be benzyl.

The preferred alkyl chain length for  $R_1$  is  $C_{12}-C_{15}$ , particularly where the alkyl group is a mixture of chain lengths  
 25 derived from coconut or palm kernel fat or is derived synthetically by olefin build up or OXO alcohols synthesis.

Preferred groups for  $R_2$ ,  $R_3$  and  $R_4$  are methyl and hydroxyethyl groups and the anion  $X$  may be selected from halide, methosulphate, acetate and phosphate ions.

30 Examples of suitable quaternary ammonium compounds of formulae (i) for use herein are:

- coconut trimethyl ammonium chloride or bromide;  
 coconut methyl dihydroxyethyl ammonium chloride or bromide;  
 decyl triethyl ammonium chloride;  
 decyl dimethyl hydroxyethyl ammonium chloride or bromide;  
 5 C<sub>12-15</sub> dimethyl hydroxyethyl ammonium chloride or bromide;  
 coconut dimethyl hydroxyethyl ammonium chloride or bromide;  
 myristyl trimethyl ammonium methyl sulphate;  
 lauryl dimethyl benzyl ammonium chloride or bromide;  
 lauryl dimethyl (ethenoxy)<sub>4</sub> ammonium chloride or bromide;  
 10 choline esters (compounds of formula (i) wherein R<sub>1</sub> is



- 15 di-alkyl imidazolines [compounds of formula (i)].

Other cationic surfactants useful herein are also described in US 4,228,044 and in EP 000 224.

When included therein, the laundry detergent compositions of the present invention typically comprise from 0.2% to about  
 20 25%, preferably from about 1% to about 8% by weight of such cationic surfactants.

Ampholytic surfactants are also suitable for use in the laundry detergent compositions of the present invention. These surfactants can be broadly described as aliphatic derivatives of  
 25 secondary or tertiary amines, or aliphatic derivatives of heterocyclic secondary and tertiary amines in which the aliphatic radical can be straight- or branched-chain. One of the aliphatic substituents contains at least about 8 carbon atoms, typically from about 8 to about 18 carbon atoms, and at least  
 30 one contains an anionic water-solubilizing group, e.g. carboxy, sulfonate, sulfate. See US 3,929,678 (column 19, lines 18-35) for examples of ampholytic surfactants.

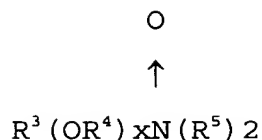
When included therein, the laundry detergent compositions of the present invention typically comprise from 0.2% to about 15%, preferably from about 1% to about 10% by weight of such ampholytic surfactants.

5        Zwitterionic surfactants are also suitable for use in laundry detergent compositions. These surfactants can be broadly described as derivatives of secondary and tertiary amines, derivatives of heterocyclic secondary and tertiary amines, or derivatives of quaternary ammonium, quaternary phosphonium or  
10 tertiary sulfonium compounds. See US 3,929,678 (column 19, line 38 through column 22, line 48) for examples of zwitterionic surfactants.

When included therein, the laundry detergent compositions of the present invention typically comprise from 0.2% to about  
15 15%, preferably from about 1% to about 10% by weight of such zwitterionic surfactants.

Semi-polar nonionic surfactants are a special category of nonionic surfactants which include water-soluble amine oxides containing one alkyl moiety of from about 10 to about 18 carbon  
20 atoms and 2 moieties selected from the group consisting of alkyl groups and hydroxyalkyl groups containing from about 1 to about 3 carbon atoms; watersoluble phosphine oxides containing one alkyl moiety of from about 10 to about 18 carbon atoms and 2 moieties selected from the group consisting of alkyl groups and  
25 hydroxyalkyl groups containing from about 1 to about 3 carbon atoms; and water-soluble sulfoxides containing one alkyl moiety from about 10 to about 18 carbon atoms and a moiety selected from the group consisting of alkyl and hydroxyalkyl moieties of from about 1 to about 3 carbon atoms.

30        Semi-polar nonionic detergent surfactants include the amine oxide surfactants having the formula:



5 wherein  $\text{R}^3$  is an alkyl, hydroxyalkyl, or alkyl phenyl group or mixtures thereof containing from about 8 to about 22 carbon atoms;  $\text{R}^4$  is an alkylene or hydroxyalkylene group containing from about 2 to about 3 carbon atoms or mixtures thereof;  $x$  is from 0 to about 3; and each  $\text{R}^5$  is an alkyl or hydroxyalkyl group  
 10 containing from about 1 to about 3 carbon atoms or a polyethylene oxide group containing from about 1 to about 3 ethylene oxide groups. The  $\text{R}^5$  groups can be attached to each other, e.g., through an oxygen or nitrogen atom, to form a ring structure.

15 These amine oxide surfactants in particular include  $\text{C}_{10}$ - $\text{C}_{18}$  alkyl dimethyl amine oxides and  $\text{C}_8$ - $\text{C}_{12}$  alkoxy ethyl dihydroxy ethyl amine oxides.

When included therein, the laundry detergent compositions of the present invention typically comprise from 0.2% to about  
 20 15%, preferably from about 1% to about 10% by weight of such semi-polar nonionic surfactants.

#### Builder system

The compositions according to the present invention may  
 25 further comprise a builder system. Any conventional builder system is suitable for use herein including aluminosilicate materials, silicates, polycarboxylates and fatty acids, materials such as ethylenediamine tetraacetate, metal ion sequestrants such as aminopolyphosphonates, particularly  
 30 ethylenediamine tetramethylene phosphonic acid and diethylene triamine pentamethylenephosphonic acid. Though less preferred for obvious environmental reasons, phosphate builders can also

be used herein.

Suitable builders can be an inorganic ion exchange material, commonly an inorganic hydrated aluminosilicate material, more particularly a hydrated synthetic zeolite such as  
5 hydrated zeolite A, X, B, HS or MAP.

Another suitable inorganic builder material is layered silicate, e.g. SKS-6 (Hoechst). SKS-6 is a crystalline layered silicate consisting of sodium silicate ( $\text{Na}_2\text{Si}_2\text{O}_5$ ).

Suitable polycarboxylates containing one carboxy group  
10 include lactic acid, glycolic acid and ether derivatives thereof as disclosed in Belgian Patent Nos. 831,368, 821,369 and 821,370. Polycarboxylates containing two carboxy groups include the water-soluble salts of succinic acid, malonic acid, (ethylenedioxy) diacetic acid, maleic acid, diglycollic acid,  
15 tartaric acid, tartronic acid and fumaric acid, as well as the ether carboxylates described in German Offenle-enschrift 2,446,686, and 2,446,487, US 3,935,257 and the sulfinyl carboxylates described in Belgian Patent No. 840,623.

Polycarboxylates containing three carboxy groups include, in  
20 particular, water-soluble citrates, aconitrates and citraconates as well as succinate derivatives such as the carboxymethyloxysuccinates described in British Patent No. 1,379,241, lactoxysuccinates described in Netherlands Application 7205873, and the oxypolycarboxylate materials such  
25 as 2-oxa-1,1,3-propane tricarboxylates described in British Patent No. 1,387,447.

Polycarboxylates containing four carboxy groups include oxydisuccinates disclosed in British Patent No. 1,261,829, 1,1,2,2,-ethane tetracarboxylates, 1,1,3,3-propane  
30 tetracarboxylates containing sulfo substituents include the sulfosuccinate derivatives disclosed in British Patent Nos. 1,398,421 and 1,398,422 and in US 3,936,448, and the sulfonated

pyrolysed citrates described in British Patent No. 1,082,179, while polycarboxylates containing phosphone substituents are disclosed in British Patent No. 1,439,000.

Alicyclic and heterocyclic polycarboxylates include  
5 cyclopentane-cis,cis-cis-tetracarboxylates, cyclopentadienide pentacarboxylates, 2,3,4,5-tetrahydro-furan - cis, cis, cis-tetracarboxylates, 2,5-tetrahydro-furan-cis, discarboxylates, 2,2,5,5,-tetrahydrofuran - tetracarboxylates, 1,2,3,4,5,6-hexane - hexacarboxylates and carboxymethyl derivatives of polyhydric  
10 alcohols such as sorbitol, mannitol and xylitol. Aromatic polycarboxylates include mellitic acid, pyromellitic acid and the phthalic acid derivatives disclosed in British Patent No. 1,425,343.

Of the above, the preferred polycarboxylates are hydroxy-  
15 carboxylates containing up to three carboxy groups per molecule, more particularly citrates.

Preferred builder systems for use in the present compositions include a mixture of a water-insoluble aluminosilicate builder such as zeolite A or of a layered  
20 silicate (SKS-6), and a water-soluble carboxylate chelating agent such as citric acid.

A suitable chelant for inclusion in the detergent compositions in accordance with the invention is ethylenediamine-N,N'-disuccinic acid (EDDS) or the alkali metal, alkaline earth  
25 metal, ammonium, or substituted ammonium salts thereof, or mixtures thereof. Preferred EDDS compounds are the free acid form and the sodium or magnesium salt thereof. Examples of such preferred sodium salts of EDDS include  $\text{Na}_2\text{EDDS}$  and  $\text{Na}_4\text{EDDS}$ . Examples of such preferred magnesium salts of EDDS include  
30  $\text{MgEDDS}$  and  $\text{Mg}_2\text{EDDS}$ . The magnesium salts are the most preferred for inclusion in compositions in accordance with the invention.

Preferred builder systems include a mixture of a water-insoluble aluminosilicate builder such as zeolite A, and a water soluble carboxylate chelating agent such as citric acid.

Other builder materials that can form part of the builder system for use in granular compositions include inorganic materials such as alkali metal carbonates, bicarbonates, silicates, and organic materials such as the organic phosphonates, amino polyalkylene phosphonates and amino polycarboxylates.

10 Other suitable water-soluble organic salts are the homo- or co-polymeric acids or their salts, in which the polycarboxylic acid comprises at least two carboxyl radicals separated from each other by not more than two carbon atoms.

Polymers of this type are disclosed in GB-A-1,596,756.  
15 Examples of such salts are polyacrylates of MW 2000-5000 and their copolymers with maleic anhydride, such copolymers having a molecular weight of from 20,000 to 70,000, especially about 40,000.

Detergency builder salts are normally included in amounts  
20 of from 5% to 80% by weight of the composition. Preferred levels of builder for liquid detergents are from 5% to 30%.

#### Enzymes:

Mannanase is incorporated into the cleaning or detergent  
25 compositions in accordance with the invention preferably at a level of from 0.0001% to 2%, more preferably from 0.0005% to 0.5%, most preferred from 0.001% to 0.1% pure enzyme by weight of the composition.

The cleaning compositions of the present invention may  
30 further comprise as an essential element a carbohydrase selected from the group consisting of cellulases, amylases, pectin degrading enzymes and xyloglucanases. Preferably, the cleaning

compositions of the present invention will comprise a mannanase, an amylase and another bioscouring-type of enzyme selected from the group consisting of cellulases, pectin degrading enzymes and xyloglucanases.

5       The cellulases usable in the present invention include both bacterial or fungal cellulases. Preferably, they will have a pH optimum of between 5 and 12 and a specific activity above 50 CEVU/mg (Cellulose Viscosity Unit). Suitable cellulases are disclosed in U.S. Patent 4,435,307, J61078384 and WO96/02653  
10 which discloses fungal cellulase produced from *Humicola insolens*, *Trichoderma*, *Thielavia* and *Sporotrichum*, respectively. EP 739 982 describes cellulases isolated from novel *Bacillus* species. Suitable cellulases are also disclosed in GB-A-2075028; GB-A-2095275; DE-OS-22 47 832 and WO95/26398.

15       Examples of such cellulases are cellulases produced by a strain of *Humicola insolens* (*Humicola grisea* var. *thermoidea*), particularly the strain *Humicola insolens*, DSM 1800. Other suitable cellulases are cellulases originated from *Humicola insolens* having a molecular weight of about 50kD, an isoelectric  
20 point of 5.5 and containing 415 amino acids; and a ~43kD endo-beta-1,4-glucanase derived from *Humicola insolens*, DSM 1800; a preferred cellulase has the amino acid sequence disclosed in PCT Patent Application No. WO 91/17243. Also suitable cellulases are the EGI III cellulases from *Trichoderma longibrachiatum* described  
25 in WO94/21801. Especially suitable cellulases are the cellulases having color care benefits. Examples of such cellulases are the cellulases described in WO96/29397, EP-A-0495257, WO 91/17243, WO91/17244 and WO91/21801. Other suitable cellulases for fabric care and/or cleaning properties are described in WO96/34092,  
30 WO96/17994 and WO95/24471.

Said cellulases are normally incorporated in the detergent composition at levels from 0.0001% to 2% of pure enzyme by



weight of the detergent composition.

Preferred cellulases for the purpose of the present invention are alkaline cellulases, i.e. enzyme having at least 25%, more preferably at least 40% of their maximum activity at a pH ranging from 7 to 12. More preferred cellulases are enzymes having their maximum activity at a pH ranging from 7 to 12. A preferred alkaline cellulase is the cellulase sold under the tradename Carezyme® by Novo Nordisk A/S.

Amylases ( $\alpha$  and/or  $\beta$ ) can be included for removal of carbohydrate-based stains. WO94/02597, Novo Nordisk A/S published February 03, 1994, describes cleaning compositions which incorporate mutant amylases. See also WO95/10603, Novo Nordisk A/S, published April 20, 1995. Other amylases known for use in cleaning compositions include both  $\alpha$ - and  $\beta$ -amylases.  $\alpha$ -Amylases are known in the art and include those disclosed in US Pat. no. 5,003,257; EP 252,666; WO/91/00353; FR 2,676,456; EP 285,123; EP 525,610; EP 368,341; and British Patent specification no. 1,296,839 (Novo). Other suitable amylases are stability-enhanced amylases described in WO94/18314, published August 18, 1994 and WO96/05295, Genencor, published February 22, 1996 and amylase variants having additional modification in the immediate parent available from Novo Nordisk A/S, disclosed in WO 95/10603, published April 95. Also suitable are amylases described in EP 277 216, WO95/26397 and WO96/23873 (all by Novo Nordisk).

Examples of commercial  $\alpha$ -amylases products are Purafect Ox Am® from Genencor and Termamyl®, Ban®, Fungamyl® and Duramyl®, all available from Novo Nordisk A/S Denmark. WO95/26397 describes other suitable amylases :  $\alpha$ -amylases characterised by having a specific activity at least 25% higher than the specific activity of Termamyl® at a temperature range of 25°C to 55°C and at a pH value in the range of 8 to 10, measured by the Phadebas

®  $\alpha$ -amylase activity assay. Suitable are variants of the above enzymes, described in WO96/23873 (Novo Nordisk). Other amylolytic enzymes with improved properties with respect to the activity level and the combination of thermostability and a higher activity level are described in WO95/35382.

Preferred amylases for the purpose of the present invention are the amylases sold under the tradename Termamyl, Duramyl and Maxamyl and or the  $\alpha$ -amylase variant demonstrating increased thermostability disclosed as SEQ ID No. 2 in WO96/23873.

10 Preferred amylases for specific applications are alkaline amylases, ie enzymes having an enzymatic activity of at least 10%, preferably at least 25%, more preferably at least 40% of their maximum activity at a pH ranging from 7 to 12. More preferred amylases are enzymes having their maximum activity at a  
15 pH ranging from 7 to 12.

The amylolytic enzymes are incorporated in the detergent compositions of the present invention a level of from 0.0001% to 2%, preferably from 0.00018% to 0.06%, more preferably from 0.00024% to 0.048% pure enzyme by weight of the composition.

20 The term "pectin degrading enzyme" is intended to encompass polygalacturonase (EC 3.2.1.15) exo-polygalacturonase (EC 3.2.1.67), exo-poly-alpha-galacturonidase (EC 3.2.1.82), pectin lyase (EC 4.2.2.10), pectin esterase (EC 3.2.1.11), pectate lyase (EC 4.2.2.2), exo-polygalacturonate lyase (EC 4.2.2.9) and  
25 hemicellulases such as endo-1,3- $\beta$ -xylosidase (EC 3.2.1.32), xylan-1,4- $\beta$ -xylosidase (EC 3.2.1.37) and  $\alpha$ -L-arabinofuranosidase (EC 3.2.1.55). The pectin degrading enzymes are natural mixtures of the above mentioned enzymatic activities. Pectin enzymes therefore include the pectin methylesterases which hydrolyse the  
30 pectin methyl ester linkages, polygalacturonases which cleave the glycosidic bonds between galacturonic acid molecules, and the pectin transeliminases or lyases which act on the pectic

acids to bring about non-hydrolytic cleavage of  $\alpha$ -1 $\rightarrow$ 4 glycosidic linkages to form unsaturated derivatives of galacturonic acid.

Pectin degrading enzymes are incorporated into the compositions in accordance with the invention preferably at a level of from 0.0001 % to 2 %, more preferably from 0.0005% to 0.5%, most preferred from 0.001 % to 0.1 % pure enzyme by weight of the total composition.

Preferred pectin degrading enzymes for specific applications are alkaline pectin degrading enzymes, ie enzymes having an enzymatic activity of at least 10%, preferably at least 25%, more preferably at least 40% of their maximum activity at a pH ranging from 7 to 12. More preferred pectin degrading enzymes are enzymes having their maximum activity at a pH ranging from 7 to 12. Alkaline pectin degrading enzymes are produced by alkalophilic microorganisms e.g. bacterial, fungal and yeast microorganisms such as *Bacillus* species. Preferred microorganisms are *Bacillus firmus*, *Bacillus circulans* and *Bacillus subtilis* as described in JP 56131376 and JP 56068393. Alkaline pectin decomposing enzymes include galacturan-1,4- $\alpha$ -galacturonase (EC 3.2.1.67), poly-galacturonase activities (EC 3.2.1.15, pectin esterase (EC 3.1.1.11), pectate lyase (EC 4.2.2.2) and their iso enzymes and they can be produced by the *Erwinia* species. Preferred are *E. chrysanthemi*, *E. carotovora*, *E. amylovora*, *E. herbicola*, *E. dissolvens* as described in JP 59066588, JP 63042988 and in World J. Microbiol. Microbiotechnol. (8, 2, 115-120) 1992. Said alkaline pectin enzymes can also be produced by *Bacillus* species as disclosed in JP 73006557 and Agr. Biol. Chem. (1972), 36(2) 285-93.

The term xyloglucanase encompasses the family of enzymes described by Vincken and Voragen at Wageningen University [Vincken et al (1994) Plant Physiol., **104**, 99-107] and are able

to degrade xyloglucans as described in Hayashi et al (1989) Plant. Physiol. Plant Mol. Biol., **40**, 139-168. Vincken et al demonstrated the removal of xyloglucan coating from cellulase of the isolated apple cell wall by a xyloglucanase purified from  
5 *Trichoderma viride* (endo-IV-glucanase). This enzyme enhances the enzymatic degradation of cell wall-embedded cellulose and work in synergy with pectic enzymes. Rapidase LIQ+ from Gist-Brocades contains an xyloglucanase activity.

This xyloglucanase is incorporated into the cleaning compo-  
10 sitions in accordance with the invention preferably at a level of from 0.0001% to 2%, more preferably from 0.0005% to 0.5%, most preferred from 0.001% to 0.1 % pure enzyme by weight of the composition.

Preferred xyloglucanases for specific applications are  
15 alkaline xyloglucanases, ie enzymes having an enzymatic activity of at least 10%, preferably at least 25%, more preferably at least 40% of their maximum activity at a pH ranging from 7 to 12. More preferred xyloglucanases are enzymes having their maximum activity at a pH ranging from 7 to 12.

20 The above-mentioned enzymes may be of any suitable origin, such as vegetable, animal, bacterial, fungal and yeast origin. Origin can further be mesophilic or extremophilic (psychrophilic, psychrotrophic, thermophilic, barophilic, alkalophilic, acidophilic, halophilic, etc.). Purified or non-  
25 purified forms of these enzymes may be used. Nowadays, it is common practice to modify wild-type enzymes via protein / genetic engineering techniques in order to optimise their performance efficiency in the cleaning compositions of the invention. For example, the variants may be designed such that the compatibility of the enzyme to commonly encountered ingredients of such  
30 compositions is increased. Alternatively, the variant may be designed such that the optimal pH, bleach or chelant stability,

catalytic activity and the like, of the enzyme variant is tailored to suit the particular cleaning application.

In particular, attention should be focused on amino acids sensitive to oxidation in the case of bleach stability and on surface charges for the surfactant compatibility. The isoelectric point of such enzymes may be modified by the substitution of some charged amino acids, e.g. an increase in isoelectric point may help to improve compatibility with anionic surfactants. The stability of the enzymes may be further enhanced by the creation of e.g. additional salt bridges and enforcing metal binding sites to increase chelant stability.

#### Bleaching agents:

Additional optional detergent ingredients that can be included in the detergent compositions of the present invention include bleaching agents such as PB1, PB4 and percarbonate with a particle size of 400-800 microns. These bleaching agent components can include one or more oxygen bleaching agents and, depending upon the bleaching agent chosen, one or more bleach activators. When present oxygen bleaching compounds will typically be present at levels of from about 1% to about 25%. In general, bleaching compounds are optional added components in non-liquid formulations, e.g. granular detergents.

A bleaching agent component for use herein can be any of the bleaching agents useful for detergent compositions including oxygen bleaches, as well as others known in the art.

A bleaching agent suitable for the present invention can be an activated or non-activated bleaching agent.

One category of oxygen bleaching agent that can be used encompasses percarboxylic acid bleaching agents and salts thereof. Suitable examples of this class of agents include magnesium monoperoxyphthalate hexahydrate, the magnesium salt of

meta-chloro perbenzoic acid, 4-nonylamino-4-oxoperoxybutyric acid and diperoxydodecanedioic acid. Such bleaching agents are disclosed in US 4,483,781, US 740,446, EP 0 133 354 and US 4,412,934. Highly preferred bleaching agents also include 6-  
5 nonylamino-6-oxoperoxypropionic acid as described in US 4,634,551.

Another category of bleaching agents that can be used encompasses the halogen bleaching agents. Examples of hypohalite bleaching agents, for example, include trichloro isocyanuric acid and the sodium and potassium dichloroisocyanurates and N-  
10 chloro and N-bromo alkane sulphonamides. Such materials are normally added at 0.5-10% by weight of the finished product, preferably 1-5% by weight.

The hydrogen peroxide releasing agents can be used in combination with bleach activators such as tetra-  
15 acetylenediamine (TAED), nonanoyloxybenzenesulfonate (NOBS, described in US 4,412,934), 3,5-trimethylhexanoyloxybenzenesulfonate (ISONOBS, described in EP 120 591) or pentaacetylglucose (PAG), which are perhydrolyzed to form a peracid as the active bleaching species, leading to improved  
20 bleaching effect. In addition, very suitable are the bleach activators C8(6-octanamido-caproyl) oxybenzene-sulfonate, C9(6-nonanamido caproyl) oxybenzenesulfonate and C10 (6-decanamido caproyl) oxybenzenesulfonate or mixtures thereof. Also suitable activators are acylated citrate esters such as disclosed in  
25 European Patent Application No. 91870207.7.

Useful bleaching agents, including peroxyacids and bleaching systems comprising bleach activators and peroxygen bleaching compounds for use in cleaning compositions according to the invention are described in application USSN 08/136,626.

30 The hydrogen peroxide may also be present by adding an enzymatic system (i.e. an enzyme and a substrate therefore) which is capable of generation of hydrogen peroxide at the

beginning or during the washing and/or rinsing process. Such enzymatic systems are disclosed in European Patent Application EP 0 537 381.

Bleaching agents other than oxygen bleaching agents are also known in the art and can be utilized herein. One type of non-oxygen bleaching agent of particular interest includes photoactivated bleaching agents such as the sulfonated zinc and/or aluminium phthalocyanines. These materials can be deposited upon the substrate during the washing process. Upon irradiation with light, in the presence of oxygen, such as by hanging clothes out to dry in the daylight, the sulfonated zinc phthalocyanine is activated and, consequently, the substrate is bleached. Preferred zinc phthalocyanine and a photoactivated bleaching process are described in US 4,033,718. Typically, detergent composition will contain about 0.025% to about 1.25%, by weight, of sulfonated zinc phthalocyanine.

Bleaching agents may also comprise a manganese catalyst. The manganese catalyst may, e.g., be one of the compounds described in "Efficient manganese catalysts for low-temperature bleaching", Nature 369, 1994, pp. 637-639.

Suds suppressors:

Another optional ingredient is a suds suppressor, exemplified by silicones, and silica-silicone mixtures. Silicones can generally be represented by alkylated polysiloxane materials, while silica is normally used in finely divided forms exemplified by silica aerogels and xerogels and hydrophobic silicas of various types. These materials can be incorporated as particulates, in which the suds suppressor is advantageously releasably incorporated in a water-soluble or water-dispersible, substantially non surface-active detergent impermeable carrier. Alternatively the suds suppressor can be dissolved or dispersed

in a liquid carrier and applied by spraying on to one or more of the other components.

A preferred silicone suds controlling agent is disclosed in US 3,933,672. Other particularly useful suds suppressors are  
5 the self-emulsifying silicone suds suppressors, described in German Patent Application DTOS 2,646,126. An example of such a compound is DC-544, commercially available from Dow Corning, which is a siloxane-glycol copolymer. Especially preferred suds controlling agent are the suds suppressor system comprising a  
10 mixture of silicone oils and 2-alkyl-alkanols. Suitable 2-alkyl-alkanols are 2-butyl-octanol which are commercially available under the trade name Isofol 12 R.

Such suds suppressor system are described in European Patent Application EP 0 593 841.

15 Especially preferred silicone suds controlling agents are described in European Patent Application No. 92201649.8. Said compositions can comprise a silicone/ silica mixture in combination with fumed nonporous silica such as Aerosil<sup>R</sup>.

The suds suppressors described above are normally employed  
20 at levels of from 0.001% to 2% by weight of the composition, preferably from 0.01% to 1% by weight.

Other components:

Other components used in detergent compositions may be  
25 employed, such as soil-suspending agents, soil-releasing agents, optical brighteners, abrasives, bactericides, tarnish inhibitors, coloring agents, and/or encapsulated or nonencapsulated perfumes.

Especially suitable encapsulating materials are water  
30 soluble capsules which consist of a matrix of polysaccharide and polyhydroxy compounds such as described in GB 1,464,616.



Other suitable water soluble encapsulating materials comprise dextrans derived from ungelatinized starch acid esters of substituted dicarboxylic acids such as described in US 3,455,838. These acid-ester dextrans are, preferably, prepared from such starches as waxy maize, waxy sorghum, sago, tapioca and potato. Suitable examples of said encapsulation materials include N-Lok manufactured by National Starch. The N-Lok encapsulating material consists of a modified maize starch and glucose. The starch is modified by adding monofunctional substituted groups such as octenyl succinic acid anhydride.

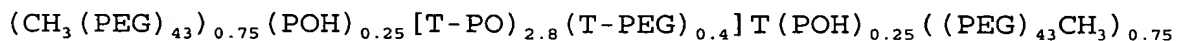
Antiredeposition and soil suspension agents suitable herein include cellulose derivatives such as methylcellulose, carboxymethylcellulose and hydroxyethylcellulose, and homo- or co-polymeric polycarboxylic acids or their salts. Polymers of this type include the polyacrylates and maleic anhydride-acrylic acid copolymers previously mentioned as builders, as well as copolymers of maleic anhydride with ethylene, methylvinyl ether or methacrylic acid, the maleic anhydride constituting at least 20 mole percent of the copolymer. These materials are normally used at levels of from 0.5% to 10% by weight, more preferably from 0.75% to 8%, most preferably from 1% to 6% by weight of the composition.

Preferred optical brighteners are anionic in character, examples of which are disodium 4,4'-bis-(2-diethanolamino-4-anilino -s- triazin-6-ylamino)stilbene-2:2' disulphonate, disodium 4, - 4'-bis-(2-morpholino-4-anilino-s-triazin-6-ylamino-stilbene-2:2' - disulphonate, disodium 4,4' - bis-(2,4-dianilino-s-triazin-6-ylamino)stilbene-2:2' - disulphonate, monosodium 4',4'' - bis-(2,4-dianilino-s-tri-azin-6-ylamino)stilbene-2-sulphonate, disodium 4,4' -bis-(2-anilino-4-(N-methyl-N-2-hydroxyethylamino) -s-triazin-6-ylamino)stilbene-2,2' - disulphonate, di-sodium 4,4' -bis-(4-phenyl-2,1,3-

triazol-2-yl)-stilbene-2,2' disulphonate, di-so-dium 4,4'bis(2-anilino-4-(1-methyl-2-hydroxyethylamino)-s-triazin-6-ylamino)stilbene-2,2'disulphonate, sodium 2(stilbyl-4''-(naphtho-1',2':4,5)-1,2,3, - triazole-2''-sulphonate and 4,4'-bis(2-sulphostyryl)biphenyl.

Other useful polymeric materials are the polyethylene glycols, particularly those of molecular weight 1000-10000, more particularly 2000 to 8000 and most preferably about 4000. These are used at levels of from 0.20% to 5% more preferably from 0.25% to 2.5% by weight. These polymers and the previously mentioned homo- or co-polymeric poly-carboxylate salts are valuable for improving whiteness maintenance, fabric ash deposition, and cleaning performance on clay, proteinaceous and oxidizable soils in the presence of transition metal impurities.

Soil release agents useful in compositions of the present invention are conventionally copolymers or terpolymers of terephthalic acid with ethylene glycol and/or propylene glycol units in various arrangements. Examples of such polymers are disclosed in US 4,116,885 and 4,711,730 and EP 0 272 033. A particular preferred polymer in accordance with EP 0 272 033 has the formula:



where PEG is  $-(\text{OC}_2\text{H}_4)_0-$ , PO is  $(\text{OC}_3\text{H}_6\text{O})$  and T is  $(\text{pOOC}_6\text{H}_4\text{CO})$ .

Also very useful are modified polyesters as random copolymers of dimethyl terephthalate, dimethyl sulfoisophthalate, ethylene glycol and 1,2-propanediol, the end groups consisting primarily of sulphobenzoate and secondarily of mono esters of ethylene glycol and/or 1,2-propanediol. The target is to obtain a polymer capped at both end by sulphobenzoate groups, "primarily", in the present context most

of said copolymers herein will be endcapped by sulphobenzoate groups. However, some copolymers will be less than fully capped, and therefore their end groups may consist of monoester of ethylene glycol and/or 1,2-propanediol, thereof consist "secondarily" of such species.

The selected polyesters herein contain about 46% by weight of dimethyl terephthalic acid, about 16% by weight of 1,2-propanediol, about 10% by weight ethylene glycol, about 13% by weight of dimethyl sulfobenzoic acid and about 15% by weight of sulfoisophthalic acid, and have a molecular weight of about 3.000. The polyesters and their method of preparation are described in detail in EP 311 342.

Softening agents:

Fabric softening agents can also be incorporated into laundry detergent compositions in accordance with the present invention. These agents may be inorganic or organic in type. Inorganic softening agents are exemplified by the smectite clays disclosed in GB-A-1 400898 and in US 5,019,292. Organic fabric softening agents include the water insoluble tertiary amines as disclosed in GB-A1 514 276 and EP 0 011 340 and their combination with mono  $C_{12}$ - $C_{14}$  quaternary ammonium salts are disclosed in EP-B-0 026 528 and di-long-chain amides as disclosed in EP 0 242 919. Other useful organic ingredients of fabric softening systems include high molecular weight polyethylene oxide materials as disclosed in EP 0 299 575 and 0 313 146.

Levels of smectite clay are normally in the range from 5% to 15%, more preferably from 8% to 12% by weight, with the material being added as a dry mixed component to the remainder of the formulation. Organic fabric softening agents such as the water-insoluble tertiary amines or dilong chain amide materials

are incorporated at levels of from 0.5% to 5% by weight, normally from 1% to 3% by weight whilst the high molecular weight polyethylene oxide materials and the water soluble cationic materials are added at levels of from 0.1% to 2%,  
5 normally from 0.15% to 1.5% by weight. These materials are normally added to the spray dried portion of the composition, although in some instances it may be more convenient to add them as a dry mixed particulate, or spray them as molten liquid on to other solid components of the composition.

10

Polymeric dye-transfer inhibiting agents:

The detergent compositions according to the present invention may also comprise from 0.001% to 10%, preferably from 0.01% to 2%, more preferably from 0.05% to 1% by weight of  
15 polymeric dye-transfer inhibiting agents. Said polymeric dye-transfer inhibiting agents are normally incorporated into detergent compositions in order to inhibit the transfer of dyes from colored fabrics onto fabrics washed therewith. These polymers have the ability of complexing or adsorbing the  
20 fugitive dyes washed out of dyed fabrics before the dyes have the opportunity to become attached to other articles in the wash.

Especially suitable polymeric dye-transfer inhibiting agents are polyamine N-oxide polymers, copolymers of N-vinyl-pyrrolidone and N-vinylimidazole, polyvinylpyrrolidone polymers,  
25 polyvinylloxazolidones and polyvinylimidazoles or mixtures thereof.

Addition of such polymers also enhances the performance of the enzymes according to the invention.

30

**Use in the paper pulp industry**

Further, it is contemplated that the mannanase of the present invention is useful in chlorine-free bleaching processes for paper pulp (chemical pulps, semichemical pulps, mechanical pulps or kraft pulps) in order to increase the brightness thereof, thus decreasing or eliminating the need for hydrogen peroxide in the bleaching process.

#### **Use in the textile and cellulosic fiber processing industries**

The mannanase of the present invention can be used in combination with other carbohydrate degrading enzymes (for instance xyloglucanase, xylanase, various pectinases) for preparation of fibers or for cleaning of fibers in combination with detergents.

In the present context, the term "cellulosic material" is intended to mean fibers, sewn and unsewn fabrics, including knits, wovens, denims, yarns, and toweling, made from cotton, cotton blends or natural or manmade cellulose (e.g. originating from xylan-containing cellulose fibers such as from wood pulp) or blends thereof. Examples of blends are blends of cotton or rayon/viscose with one or more companion material such as wool, synthetic fibers (e.g. polyamide fibers, acrylic fibers, polyester fibers, polyvinyl alcohol fibers, polyvinyl chloride fibers, polyvinylidene chloride fibers, polyurethane fibers, polyurea fibers, aramid fibers), and cellulose-containing fibers (e.g. rayon/viscose, ramie, hemp, flax/linen, jute, cellulose acetate fibers, lyocell).

The processing of cellulosic material for the textile industry, as for example cotton fiber, into a material ready for garment manufacture involves several steps: spinning of the fiber into a yarn; construction of woven or knit fabric from the yarn and subsequent preparation, dyeing and finishing operations. Woven goods are constructed by weaving a filling yarn between a series of warp yarns; the yarns could be two

different types.

Desizing: polymeric size like e.g. mannan, starch, CMC or PVA is added before weaving in order to increase the warp speed; This material must be removed before further processing. The  
5 enzyme of the invention is useful for removal of mannan containing size.

#### **Degradation of thickeners**

Galactomannans such as guar gum and locust bean gum are  
10 widely used as thickening agents e.g. in food and print paste for textile printing such as prints on T-shirts. The enzyme or enzyme preparation according to the invention can be used for reducing the viscosity of eg residual food in processing equipment and thereby facilitate cleaning after processing. Further,  
15 it is contemplated that the enzyme or enzyme preparation is useful for reducing viscosity of print paste, thereby facilitating wash out of surplus print paste after textile printins.

#### **Degradation or modification of plant material**

20 The enzyme or enzyme preparation according to the invention is preferably used as an agent for degradation or modification of mannan, galactomannan, glucomannan or galactoglucomannan containing material originating from plants. Examples of such material is guar gum and locust bean gum.

25 The mannanase of the invention may be used in modifying the physical-chemical properties of plant derived material such as the viscosity. For instance, the mannanase may be used to reduce the viscosity of feed or food which contain mannan and to promote processing of viscous mannan containing material.

30

#### **Coffee extraction**

The enzyme or enzyme preparation of the invention may also be used for hydrolysing galactomannans present in a liquid coffee extract, preferably in order to inhibit gel formation during freeze drying of the (instant) coffee. Preferably, the mannanase  
5 of the invention is immobilized in order to reduce enzyme consumption and avoid contamination of the coffee. This use is further disclosed in EP-A-676 145.

## **MATERIALS AND METHODS**

### **10 Assay for activity test**

A polypeptide of the invention having mannanase activity may be tested for mannanase activity according to standard test procedures known in the art, such as by applying a solution to be tested to 4 mm diameter holes punched out in agar plates  
15 containing 0.2% AZCL galactomannan (carob), i.e. substrate for the assay of endo-1,4-beta-D-mannanase available as CatNo.I-AZGMA from the company Megazyme (Megazyme's Internet address: <http://www.megazyme.com/Purchase/index.html>).

### **20 Determination of catalytic activity (ManU) of mannanase** **Colorimetric Assay**

Substrate: 0.2% AZCL-Galactomannan (Megazyme, Australia) from carob in 0.1 M Glycin buffer, pH 10.0.

The assay is carried out in an Eppendorf Micro tube 1.5 ml  
25 on a thermomixer with stirring and temperature control of 40°C. Incubation of 0.750 ml substrate with 0.05 ml enzyme for 20 min, stop by centrifugation for 4 minutes at 15000 rpm. The colour of the supernatant is measured at 600 nm in a 1 cm cuvette.

30 One ManU (Mannanase units) gives 0.24 abs in 1 cm.

### Strains and donor organism

The *Bacillus halodurans* mentioned above comprises the beta-1,4-mannanase encoding DNA sequence shown in SEQ.ID.NO:1.

*E.coli* DSM 12441 comprises the plasmid containing the DNA  
5 encoding the beta-1,4-mannanase of the invention (SEQ.ID.NO:1).

*E. coli* strain: Cells of *E. coli* SJ2 (Diderichsen, B., Wedsted, U., Hedegaard, L., Jensen, B. R., Sjøholm, C. (1990) Cloning of *aldB*, which encodes alpha-acetolactate decarboxylase, an exoenzyme from *Bacillus brevis*. *J. Bacteriol.*, 172, 4315-  
10 4321), were prepared for and transformed by electroporation using a Gene Pulser™ electroporator from BIO-RAD as described by the supplier.

*B.subtilis* PL2306. This strain is the *B.subtilis* DN1885 with disrupted *apr* and *npr* genes (Diderichsen, B., Wedsted, U.,  
15 Hedegaard, L., Jensen, B. R., Sjøholm, C. (1990) Cloning of *aldB*, which encodes alpha-acetolactate decarboxylase, an exoenzyme from *Bacillus brevis*. *J. Bacteriol.*, 172, 4315-4321) disrupted in the transcriptional unit of the known *Bacillus subtilis* cellulase gene, resulting in cellulase negative cells. The  
20 disruption was performed essentially as described in ( Eds. A.L. Sonenshein, J.A. Hoch and Richard Losick (1993) *Bacillus subtilis* and other Gram-Positive Bacteria, American Society for microbiology, p.618).

Competent cells were prepared and transformed as described  
25 by Yasbin, R.E., Wilson, G.A. and Young, F.E. (1975) Transformation and transfection in lysogenic strains of *Bacillus subtilis*: evidence for selective induction of prophage in competent cells. *J. Bacteriol.*, 121:296-304.

### 30 General molecular biology methods:

Unless otherwise stated all the DNA manipulations and



transformations were performed using standard methods of molecular biology (Sambrook et al. (1989) Molecular cloning: A laboratory manual, Cold Spring Harbor lab., Cold Spring Harbor, NY; Ausubel, F. M. et al. (eds.) "Current protocols in Molecular Biology". John Wiley and Sons, 1995; Harwood, C. R., and Cutting, S. M. (eds.) "Molecular Biological Methods for Bacillus". John Wiley and Sons, 1990).

Enzymes for DNA manipulations were used according to the manufacturer's instructions (e.g. restriction endonucleases, ligases etc. are obtainable from New England Biolabs, Inc.).

### Plasmids

**pSJ1678:** (see International Patent Application published as WO 94/19454).

15 **pBK-CMV** (Stratagene inc., La Jolla Ca.)

**pMOL944.** This plasmid is a pUB110 derivative essentially containing elements making the plasmid propagatable in *Bacillus subtilis*, kanamycin resistance gene and having a strong promoter and signal peptide cloned from the amyL gene of *B.licheniformis* ATCC14580. The signal peptide contains a SacII site making it convenient to clone the DNA encoding the mature part of a protein in-fusion with the signal peptide. This results in the expression of a Pre-protein which is directed towards the exterior of the cell.

25 The plasmid was constructed by means of ordinary genetic engineering and is briefly described in the following.

#### Construction of pMOL944:

The pUB110 plasmid (McKenzie, T. et al., 1986, Plasmid 15:93-103) was digested with the unique restriction enzyme NciI. A PCR fragment amplified from the amyL promoter encoded on the plasmid pDN1981 (P.L. Jørgensen et al., 1990, Gene, 96, p37-41.)

was digested with NciI and inserted in the NciI digested pUB110 to give the plasmid pSJ2624.

The two PCR primers used have the following sequences:

# LWN5494 5'-GTCGCCGGGGCGGCCGCTATCAATTGGTAACTGTATCTCAGC -3'  
 5 # LWN5495 5'-GTCGCCCGGGAGCTCTGATCAGGTACCAAGCTTGTTCGACCTGCAGAA  
 TGAGGCAGCAAGAAGAT -3'

The primer #LWN5494 inserts a NotI site in the plasmid.

The plasmid pSJ2624 was then digested with SacI and NotI and a new PCR fragment amplified on amyL promoter encoded on the  
 10 pDN1981 was digested with SacI and NotI and this DNA fragment was inserted in the SacI-NotI digested pSJ2624 to give the plasmid pSJ2670.

This cloning replaces the first amyL promoter cloning with the same promoter but in the opposite direction. The two primers  
 15 used for PCR amplification have the following sequences:

#LWN5938 5'-GTCGGCGGCCGCTGATCACGTACCAAGCTTGTTCGACCTGCAGAATG  
 AGGCAGCAAGAAGAT -3'  
 #LWN5939 5'-GTCGGAGCTCTATCAATTGGTAACTGTATCTCAGC -3'

20

The plasmid pSJ2670 was digested with the restriction enzymes PstI and BclI and a PCR fragment amplified from a cloned DNA sequence encoding the alkaline amylase SP722 (Patent # WO9526397-A1) was digested with PstI and BclI and inserted to  
 25 give the plasmid pMOL944. The two primers used for PCR amplification have the following sequence:

#LWN7864 5' -AACAGCTGATCACGACTGATCTTTTAGCTTGGCAC-3'  
 #LWN7901 5' -AACTGCAGCCGCGGCACATCATAATGGGACAAATGGG -3'

The primer #LWN7901 inserts a SacII site in the plasmid.

30

**Cultivation of donor strains and isolation of genomic DNA**

The *Bacillus halodurans* was grown in TY with pH adjusted to approximately pH 9.7 by the addition of 50 ml of 1M Sodium-Sesquicarbonat per 500 ml TY. After 24 hours incubation at 30°C and 300 rpm, the cells were harvested, and genomic DNA was  
5 isolated by the method described by Pitcher et al. [Pitcher, D. G., Saunders, N. A., Owen, R. J; Rapid extraction of bacterial genomic DNA with guanidium thiocyanate; Lett Appl Microbiol 1989 8 151-156].

10 **Construction of a genomic library from *Bacillus halodurans* in the pSJ1678 vector**

Genomic DNA of *Bacillus halodurans* was partially digested with restriction enzyme *Sau3A*, and size-fractionated by electrophoresis on a 0.7 % agarose gel (SeaKem agarose, FMC, USA).  
15 DNA fragments between 2 and 10 kb in size was isolated by electrophoresis onto DEAE-cellulose paper (Dretzen, G., Bellard, M., Sassone-Corsi, P., Chambon, P. (1981) A reliable method for the recovery of DNA fragments from agarose and acrylamide gels. Anal. Biochem., 112, 295-298). Isolated DNA fragments were  
20 ligated to *Bam*HI-digested pSJ1678 plasmid DNA, and the ligation mixture was used to transform *E. coli* SJ2.

**Screening for beta-mannanase clones by functional expression in *Escherichia coli***

25 Approximately 10.000 colony-forming units (cfu) from the genomic library were plated on LB-agar plates containing containing 9 µg/ml chloramphenicol and 0.1 % AZCL-galactomannan (MegaZyme, Australia, cat. no. I-AZGMA), using *E. coli* SJ2 as a host, followed by incubation of the plates at 37°C for 24 hours.  
30 Mannanase-positive *E. coli* colonies were identified by the formation of blue hydrolysis halos around the positive plasmid clones. The mannanase-positive clones in pSJ1678 were colony-

purified by re-streaking the isolated colonies on LB plates containing 9 µg/ml Chloramphenicol and 0.1 % AZCL-galactomannan as above. Single, mannanase-positive plasmid clones were inoculated into 5 ml of LB medium containing containing 9 µg/ml  
5 Chloramphenicol, for purification of the plasmid DNA.

### **Nucleotide sequence analysis**

The nucleotide sequence of the genomic beta-1,4-mannanase clone pBXM5 was determined from both strands by the dideoxy  
10 chain-termination method (Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463-5467) using 500 ng of Qiagen-purified template (Qiagen, USA), the Taq deoxy-terminal cycle sequencing kit (Perkin-Elmer, USA), fluorescent labeled terminators and 5 pmol of either pBK-CMV  
15 polylinker primers (Stratagene, USA) or synthetic oligonucleotide primers. Analysis of the sequence data was performed according to Devereux et al., 1984 (Devereux, J., Haeberli, P., and Smithies, O. (1984) Nucleic Acids Res. 12, 387-395).

### **20 Sequence alignment**

A multiple sequence alignment of the glycohydrolase family  
5 beta-1,4-mannanase from *Bacillus halodurans* of the present invention (ie SEQ ID NO:2), *Bacillus circulans* (GenBank/EMBL accession no. 066185), *Vibrio sp.* (acc. no. 069347),  
25 *Streptomyces lividans* (acc. no. P51529), and *Caldicellulosiruptor saccharolyticus* (acc. no. P22533). The multiple sequence alignment was created using the PileUp program of the GCG Wisconsin software package, version 8.1.; with gap creation penalty 3.00 and gap extension penalty 0.10.

30

### Sequence Similarities:

The deduced amino acid sequence of the family 5 beta-1,4-mannanase of the present invention cloned from *Bacillus halodurans* shows 77% similarity and 60% sequence identity to the beta-1,4-mannanase of *Bacillus circulans* (GenBank/EMBL accession no. O66185), 64.2% similarity and 46% identity to the beta-1,4-mannanase from *Vibrio sp.* (acc. no. O69347), 63% similarity and 41.8% identity to the beta-1,4-mannanase from *Streptomyces lividans* (acc. no. P51529), 60.3% similarity and 42% sequence identity to the beta-1,4-mannanase from *Caldicellulosiruptor saccharolyticus* (acc. no. P2253). The sequences were aligned using the GAP program of the GCG Wisconsin software package, version 8.1.; with gap creation penalty 3.00 and gap extension penalty 0.10.

#### 15 **Media**

**TY** (as described in Ausubel, F. M. et al. (eds.) "Current protocols in Molecular Biology". John Wiley and Sons, 1995).

**LB agar** (as described in Ausubel, F. M. et al. (eds.) "Current protocols in Molecular Biology". John Wiley and Sons, 20 1995).

**LBPG** is LB agar supplemented with 0.5% Glucose and 0.05 M potassium phosphate, pH 7.0

**AZCL-galactomannan** is added to LBPG-agar to 0.5 % AZCL-galactomannan is from Megazyme, Australia.

25 **BPX media** is described in EP 0 506 780 (WO 91/09129).

**NZY agar** (per liter) 5 g of NaCl, 2 g of MgSO<sub>4</sub>, 5 g of yeast extract, 10 g of NZ amine (casein hydrolysate), 15 g of agar; add deionized water to 1 liter, adjust pH with NaOH to pH 7.5 and autoclave

30 **NZY broth** (per liter) 5 g of NaCl, 2 g of MgSO<sub>4</sub>, 5 g of yeast extract, 10 g of NZ amine (casein hydrolysate); add deionized water to 1 liter, adjust pH with NaOH to pH 7.5 and auto-

clave

**NZY Top Agar** (per liter) 5 g of NaCl, 2 g of MgSO<sub>4</sub>, 5 g of yeast extract, 10 g of NZ amine (casein hydrolysate), 0.7 % (w/v) agarose; add deionized water to 1 liter, adjust pH with NaOH to pH 7.5 and autoclave.

The following examples illustrate the invention.

#### EXAMPLE 1

##### 10 Cloning of *Bacillus halodurans* mannanase gene

Subcloning and expression of mature full length mannanase in *B.subtilis*

The mannanase encoding DNA sequence of the invention was PCR amplified using the PCR primer set consisting of these two oligo nucleotides:

BXM5.upper.SacII

5'-CAT TCT GCA GCC GCG GCA CAT CAC AGT GGG TTC CAT G-3'

20 BXM5.lower.NotI

5'-GCG TTG AGA CGC GCG GCC GCT TAT TGA AAC ACA CTG CTT CTT TTA G-3'

Restriction sites SacII and NotI are underlined

Chromosomal DNA isolated from *Bacillus halodurans* as described above was used as template in a PCR reaction using Amplitaq DNA Polymerase (Perkin Elmer) according to manufacturers instructions. The PCR reaction was set up in PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 0.01 % (w/v) gelatin) containing 200  $\mu$ M of each dNTP, 2.5 units of AmpliTa<sub>q</sub> polymerase (Perkin-Elmer, Cetus, USA) and 100 pmol of each primer.

The PCR reactions was performed using a DNA thermal cycler (Landgraf, Germany). One incubation at 94°C for 1 min followed by thirty cycles of PCR performed using a cycle profile of denaturation at 94°C for 30 sec, an-nea-ling at 60°C for 1 min, and extension at 72°C for 2 min. Five- $\mu$ l aliquots of the ampli-fication product was analysed by electrophoresis in 0.7 % agarose gels (NuSieve, FMC). The appearance of a DNA fragment size 0.9 kb indicated proper amplification of the gene segment.

10 Subcloning of PCR fragment:

Fortyfive- $\mu$ l aliquots of the PCR products generated as described above were purified using QIA-quick PCR purification kit (Qiagen, USA) according to the manufacturer's instructions. The purified D-NA was eluted in 50  $\mu$ l of 10mM Tris-HCl, pH 8.5.

15 5  $\mu$ g of pMOL944 and twentyfive- $\mu$ l of the purified PCR fragment was digested with SacII and NotI, electrophoresed in 0.8 % low gelling temperature agarose (SeaPla-que GTG, FMC) gels, the relevant fragments were excised from the gels, and purified using QIA-quick Gel extraction Kit (Qiagen, USA) according to  
20 the manufacturer's instructions. The isolated PCR DNA fragment was then ligated to the SacII-NotI digested and purified pMOL944. The ligation was performed overnight at 16°C using 0.5  $\mu$ g of each DNA fragment, 1 U of T4 DNA ligase and T4 ligase buffer (Boehringer Mannheim, Germany).

25 The ligation mixture was used to transform competent B.subtilis PL2306. The transformed cells were plated onto LBPG-10  $\mu$ g/ml of Kanamycin-agar plates. After 18 hours incubation at 37°C colonies were seen on plates. Several clones were analyzed by isolating plasmid DNA from overnight culture broth.

30 One such positive clone was restreaked several times on agar plates as used above, this clone was called MB878. The clone MB878 was grown overnight in TY-10 $\mu$ g/ml Kanamycin at 37°C,

and next day 1 ml of cells were used to isolate plasmid from the cells using the Qiaprep Spin Plasmid Miniprep Kit #27106 according to the manufacturers recommendations for B.subtilis plasmid preparations. This DNA was DNA sequenced and revealed the DNA  
5 sequence corresponding to the mature part of the mannanase position 138-1034 in SEQ ID NO. 1 and 33-331 in the SEQ ID NO. 2.

## EXAMPLE 2

### 10 **Expression, purification and characterisation of mannanase from *Bacillus halodurans***

The clone MB878 obtained as described above in Example 1 was grown in 25 x 200ml BPX media with 10 µg/ml of Kanamycin in 500ml two baffled shakeflasks for 5 days at 37°C at 300 rpm.

15 5000 ml of the shake flask culture fluid of the clone MB878 was collected and pH was adjusted to 6.0. 125 ml of cationic agent (10% C521) and 250 ml of anionic agent (A130) was added during agitation for flocculation. The flocculated material was separated by centrifugation using a Sorval RC 3B centrifuge at  
20 9000 rpm for 20 min at 6°C. The supernatant was adjusted to pH 8.0 using NaOH and clarified using Whatman glass filters GF/D and C. Then 50 g of DEAE A-50 Sephadex was equilibrated with 0.1M Sodium acetate, pH 6.0, and added to the filtrate, the enzyme was bound and left overnight at room temperature. The  
25 bound enzyme was eluted with 0.5 M NaCl in the acetate buffer. Then the pH was adjusted to pH 8.0 using sodium hydroxide and then concentrated on a Filtron with a 10 kDa cut off to 450 ml and then stabilized with 20% glycerol, 20% MPG and 2% Berol. The product was used for application trials.

30 2 ml of this concentrate was adjusted to pH 8.5 using sodium hydroxide. For obtaining highly pure mannanase the concentrate was passed over a Superdex column equilibrated with 0.1 M



sodium phosphate, pH 8.5.

The pure enzyme gave a single band in SDS-PAGE with a molecular weight of 34 kDa.

The amino acid sequence of the mannanase enzyme, i.e. the translated DNA sequence, is shown in SEQ ID NO:2.

The following N-terminal sequence of the purified protein was determined: AHHSGFHVNGTTLYDA.

The pH activity profile using the ManU assay (incubation for 20 minutes at 40°C) shows that the enzyme has a relative activity higher than 50% between pH 7.5 and pH 10.

Temperature optimum was found (using the ManU assay; glycine buffer) to be between 60°C and 70°C at pH 10.

Immunological properties: Rabbit polyclonal monospecific serum was raised against the highly purified cloned mannanase using conventional techniques at the Danish company DAKO. The serum formed a nice single precipitate in agarose gels with the crude non purified mannanase of the invention.

### EXAMPLE 3

#### 20 Use of the enzyme of the invention in detergents

Using commercial detergents instead of buffer and incubation for 20 minutes at 40°C with 0.2% AZCL-Galactomannan (Megazyme, Australia) from carob degree as described above followed by determination of the formation of blue color, the enzyme obtained as described in example 2 was active with an activity higher than 40% relative to the activity in buffer in European liquid detergent Ariel Futur, in US Tide powder and in US Tide liquid detergent. In these tests, the detergent concentration was as recommended on the commercial detergent packages and the wash water was tap water having 18 degrees German hardness under European (Ariel Futur) conditions and 9 degree under US conditions (US Tide).

**EXAMPLES 4-28**

The following examples are meant to exemplify compositions of the present invention, but are not necessarily meant to limit 5 or otherwise define the scope of the invention.

In the detergent compositions, the enzymes levels are expressed by pure enzyme by weight of the total composition and unless otherwise specified, the detergent ingredients are expressed by weight of the total compositions. The abbreviated 10 component identifications therein have the following meanings:

LAS	: Sodium linear C <sub>11-13</sub> alkyl benzene sulphonate.
TAS	: Sodium tallow alkyl sulphate.
CxyAS	: Sodium C <sub>1x</sub> - C <sub>1y</sub> alkyl sulfate.
CxySAS	: Sodium C <sub>1x</sub> - C <sub>1y</sub> secondary (2,3) alkyl sulfate.
CxyEz	: C <sub>1x</sub> - C <sub>1y</sub> predominantly linear primary alcohol condensed with an average of z moles of ethylene oxide.
CxyEzS	: C <sub>1x</sub> - C <sub>1y</sub> sodium alkyl sulfate condensed with an average of z moles of ethylene oxide.
QAS	: R <sub>2</sub> .N+(CH <sub>3</sub> ) <sub>2</sub> (C <sub>2</sub> H <sub>4</sub> OH) with R <sub>2</sub> = C <sub>12</sub> -C <sub>14</sub> .
QAS 1	: R <sub>2</sub> .N+(CH <sub>3</sub> ) <sub>2</sub> (C <sub>2</sub> H <sub>4</sub> OH) with R <sub>2</sub> = C <sub>8</sub> -C <sub>11</sub> .
APA	: C <sub>8-10</sub> amido propyl dimethyl amine.
Soap	: Sodium linear alkyl carboxylate derived from a 80/20 mixture of tallow and coconut fatty acids.

Nonionic	: C <sub>13</sub> -C <sub>15</sub> mixed ethoxylated/propoxylated fatty alcohol with an average degree of ethoxylation of 3.8 and an average degree of propoxylation of 4.5.
Neodol 45-13	: C <sub>14</sub> -C <sub>15</sub> linear primary alcohol ethoxylate, sold by Shell Chemical CO.
STS	: Sodium toluene sulphonate.
CFAA	: C <sub>12</sub> -C <sub>14</sub> alkyl N-methyl glucamide.
TFAA	: C <sub>16</sub> -C <sub>18</sub> alkyl N-methyl glucamide.
TPKFA	: C <sub>12</sub> -C <sub>14</sub> topped whole cut fatty acids.
Silicate	: Amorphous Sodium Silicate (SiO <sub>2</sub> :Na <sub>2</sub> O ratio = 1.6-3.2).
Metasilicate	: Sodium metasilicate (SiO <sub>2</sub> :Na <sub>2</sub> O ratio = 1.0).
Zeolite A	: Hydrated Sodium Aluminosilicate of formula Na <sub>12</sub> (AlO <sub>2</sub> SiO <sub>2</sub> ) <sub>12</sub> . 27H <sub>2</sub> O having a primary particle size in the range from 0.1 to 10 micrometers (Weight expressed on an anhydrous basis).
Na-SKS-6	: Crystalline layered silicate of formula $\delta$ -Na <sub>2</sub> Si <sub>2</sub> O <sub>5</sub> .
Citrate	: Tri-sodium citrate dihydrate of activity 86.4% with a particle size distribution between 425 and 850 micrometres.
Citric	: Anhydrous citric acid.
Borate	: Sodium borate
Carbonate	: Anhydrous sodium carbonate with a particle size between 200 and 900 micrometres.

Bicarbonate	: Anhydrous sodium hydrogen carbonate with a particle size distribution between 400 and 1200 micrometres.
Sulphate	: Anhydrous sodium sulphate.
Mg Sulphate	: Anhydrous magnesium sulfate.
STPP	: Sodium tripolyphosphate.
TSPP	: Tetrasodium pyrophosphate.
MA/AA	: Random copolymer of 4:1 acrylate/maleate, average molecular weight about 70,000-80,000.
MA/AA 1	: Random copolymer of 6:4 acrylate/maleate, average molecular weight about 10,000.
AA	: Sodium polyacrylate polymer of average molecular weight 4,500.
PA30	: Polyacrylic acid of average molecular weight of between about 4,500 - 8,000.
480N	: Random copolymer of 7:3 acrylate/methacrylate, average molecular weight about 3,500.
Polygel/carbopo 1	: High molecular weight crosslinked polyacrylates.
PB1	: Anhydrous sodium perborate monohydrate of nominal formula $\text{NaBO}_2 \cdot \text{H}_2\text{O}_2$ .
PB4	: Sodium perborate tetrahydrate of nominal formula $\text{NaBO}_2 \cdot 3\text{H}_2\text{O} \cdot \text{H}_2\text{O}_2$ .
Percarbonate	: Anhydrous sodium percarbonate of nominal formula $2\text{Na}_2\text{CO}_3 \cdot 3\text{H}_2\text{O}_2$ .
NaDCC	: Sodium dichloroisocyanurate.
TAED	: Tetraacetylenediamine.
NOBS	: Nonanoyloxybenzene sulfonate in the form of the sodium salt.

NACA-OBS	: (6-nonamidocaproyl) oxybenzene sulfonate.
DTPA	: Diethylene triamine pentaacetic acid.
HEDP	: 1,1-hydroxyethane diphosphonic acid.
DETPMP	: Diethyltriamine penta (methylene) phosphonate, marketed by Monsanto under the Trade name Dequest 2060.
EDDS	: Ethylenediamine-N,N'-disuccinic acid, (S,S) isomer in the form of its sodium salt
MnTACN	: Manganese 1,4,7-trimethyl-1,4,7-triazacyclononane.
Photoactivated Bleach	: Sulfonated zinc phthalocyanine encapsulated in dextrin soluble polymer.
Photoactivated Bleach 1	: Sulfonated alumino phthalocyanine encapsulated in dextrin soluble polymer.
PAAC	: Pentaamine acetate cobalt(III) salt.
Paraffin	: Paraffin oil sold under the tradename Winog 70 by Wintershall.
NaBz	: Sodium benzoate.
BzP	: Benzoyl Peroxide.
Mannanase	: As described herein
Protease	: Proteolytic enzyme sold under the trade-name Savinase, Alcalase, Durazym by Novo Nordisk A/S, Maxacal, Maxapem sold by Gist-Brocades and proteases described in patents WO91/06637 and/or WO95/10591 and/or EP 251 446.

- Amylase : Amylolytic enzyme sold under the tradename Purafact Ox Am<sup>R</sup> described in WO 94/18314, WO96/05295 sold by Genencor; Termamyl<sup>®</sup>, Fungamyl<sup>®</sup> and Duramyl<sup>®</sup>, all available from Novo Nordisk A/S and those described in WO95/26397.
- Lipase : Lipolytic enzyme sold under the tradename Lipolase, Lipolase Ultra by Novo Nordisk A/S and Lipomax by Gist-Brocades.
- Cellulase : Cellulytic enzyme sold under the tradename Carezyme, Celluzyme and/or Endolase by Novo Nordisk A/S.
- CMC : Sodium carboxymethyl cellulose.
- PVP : Polyvinyl polymer, with an average molecular weight of 60,000.
- PVNO : Polyvinylpyridine-N-Oxide, with an average molecular weight of 50,000.
- PVPVI : Copolymer of vinylimidazole and vinylpyrrolidone, with an average molecular weight of 20,000.
- Brightener 1 : Disodium 4,4'-bis(2-sulphostyryl)biphenyl.
- Brightener 2 : Disodium 4,4'-bis(4-anilino-6-morpholino-1.3.5-triazin-2-yl) stilbene-2:2'-disulfonate.
- Silicone anti-foam : Polydimethylsiloxane foam controller with siloxane-oxyalkylene copolymer as dispersing agent with a ratio of said foam controller to said dispersing agent of 10:1 to 100:1.
- Suds Suppressor : 12% Silicone/silica, 18% stearyl alcohol, 70% starch in granular form.

Opacifier : Water based monostyrene latex mixture,  
sold by BASF Aktiengesellschaft under the  
tradename Lytron 621.

SRP 1 : Anionically end capped poly esters.

SRP 2 : Diethoxylated poly (1,2 propylene tere-  
phthalate) short block polymer.

QEA : bis((C<sub>2</sub>H<sub>5</sub>O)(C<sub>2</sub>H<sub>4</sub>O)<sub>n</sub>)(CH<sub>3</sub>) -N<sup>+</sup>-C<sub>6</sub>H<sub>12</sub>-N<sup>+</sup>-  
(CH<sub>3</sub>) bis((C<sub>2</sub>H<sub>5</sub>O)-(C<sub>2</sub>H<sub>4</sub>O))<sub>n</sub>, wherein n =  
from 20 to 30.

PEI : Polyethyleneimine with an average molecu-  
lar weight of 1800 and an average ethoxy-  
lation degree of 7 ethyleneoxy residues  
per nitrogen.

SCS : Sodium cumene sulphonate.

HMWPEO : High molecular weight polyethylene oxide.

PEGx : Polyethylene glycol, of a molecular weight  
of x .

PEO : Polyethylene oxide, with an average mo-  
lecular weight of 5,000.

TEPAE : Tetraethylenepentaamine ethoxylate.

BTA : Benzotriazole.

PH : Measured as a 1% solution in distilled  
water at 20°C.

Example 4

The following high density laundry detergent compositions were prepared according to the present invention :

	I	II	III	IV	V	VI
LAS	8.0	8.0	8.0	2.0	6.0	6.0
TAS	-	0.5	-	0.5	1.0	0.1
C46(S)AS	2.0	2.5	-	-	-	-
C25AS	-	-	-	7.0	4.5	5.5
C68AS	2.0	5.0	7.0	-	-	-
C25E5	-	-	3.4	10.0	4.6	4.6
C25E7	3.4	3.4	1.0	-	-	-
C25E3S	-	-	-	2.0	5.0	4.5
QAS	-	0.8	-	-	-	-
QAS 1	-	-	-	0.8	0.5	1.0
Zeolite A	18.1	18.0	14.1	18.1	20.0	18.1
Citric	-	-	-	2.5	-	2.5
Carbonate	13.0	13.0	27.0	10.0	10.0	13.0
Na-SKS-6	-	-	-	10.0	-	10.0
Silicate	1.4	1.4	3.0	0.3	0.5	0.3
Citrate	-	1.0	-	3.0	-	-
Sulfate	26.1	26.1	26.1	6.0	-	-
Mg sulfate	0.3	-	-	0.2	-	0.2
MA/AA	0.3	0.3	0.3	4.0	1.0	1.0
CMC	0.2	0.2	0.2	0.2	0.4	0.4
PB4	9.0	9.0	5.0	-	-	-
Percarbonate	-	-	-	-	18.0	18.0
TAED	1.5	0.4	1.5	-	3.9	4.2
NACA-OBS	-	2.0	1.0	-	-	-
DETPMP	0.25	0.25	0.25	0.25	-	-
SRP 1	-	-	-	0.2	-	0.2



	I	II	III	IV	V	VI
EDDS	-	0.25	0.4	-	0.5	0.5
CFAA	-	1.0	-	2.0	-	-
HEDP	0.3	0.3	0.3	0.3	0.4	0.4
QEA	-	-	-	0.2	-	0.5
Protease	0.009	0.009	0.01	0.04	0.05	0.03
Mannanase	0.05	0.009	0.03	0.009	0.03	0.009
Amylase	0.002	0.002	0.002	0.006	0.008	0.008
Cellulase	0.0007	-	-	0.0007	0.0007	0.0007
Lipase	0.006	-	-	0.01	0.01	0.01
Photoactivated bleach (ppm)	15	15	15	-	20	20
PVNO/PVPVI	-	-	-	0.1	-	-
Brightener 1	0.09	0.09	0.09	-	0.09	0.09
Perfume	0.3	0.3	0.3	0.4	0.4	0.4
Silicone anti- foam	0.5	0.5	0.5	-	0.3	0.3
Density in g/litre	850	850	850	850	850	850
Miscellaneous and minors	Up to 100%					

Example 5

The following granular laundry detergent compositions of particular utility under European machine wash conditions were prepared according to the present invention :

5

	I	II	III	IV	V	VI
LAS	5.5	7.5	5.0	5.0	6.0	7.0
TAS	1.25	1.9	-	0.8	0.4	0.3
C24AS/C25AS	-	2.2	5.0	5.0	5.0	2.2
C25E3S	-	0.8	1.0	1.5	3.0	1.0
C45E7	3.25	-	-	-	-	3.0
TFAA	-	-	2.0	-	-	-
C25E5	-	5.5	-	-	-	-
QAS	0.8	-	-	-	-	-
QAS 1	-	0.7	1.0	0.5	1.0	0.7
STPP	19.7	-	-	-	-	-
Zeolite A	-	19.5	25.0	19.5	20.0	17.0
NaSKS-6/citric acid (79:21)	-	10.6	-	10.6	-	-
Na-SKS-6	-	-	9.0	-	10.0	10.0
Carbonate	6.1	21.4	9.0	10.0	10.0	18.0
Bicarbonate	-	2.0	7.0	5.0	-	2.0
Silicate	6.8	-	-	0.3	0.5	-
Citrate	-	-	4.0	4.0	-	-
Sulfate	39.8	-	-	5.0	-	12.0
Mg sulfate	-	-	0.1	0.2	0.2	-
MA/AA	0.5	1.6	3.0	4.0	1.0	1.0
CMC	0.2	0.4	1.0	1.0	0.4	0.4
PB4	5.0	12.7	-	-	-	-
Percarbonate	-	-	-	-	18.0	15.0
TAED	0.5	3.1	-	-	5.0	-

	I	II	III	IV	V	VI
NACA-OBS	1.0	3.5	-	-	-	2.5
DETPMP	0.25	0.2	0.3	0.4	-	0.2
HEDP	-	0.3	-	0.3	0.3	0.3
QEA	-	-	1.0	1.0	1.0	-
Protease	0.009	0.03	0.03	0.05	0.05	0.02
Mannanase	0.03	0.03	0.001	0.03	0.005	0.009
Lipase	0.003	0.003	0.006	0.006	0.006	0.004
Cellulase	0.000	0.000	0.000	0.000	0.000	0.000
	6	6	5	5	7	7
Amylase	0.002	0.002	0.006	0.006	0.01	0.003
PVNO/PVPVI	-	-	0.2	0.2	-	-
PVP	0.9	1.3	-	-	-	0.9
SRP 1	-	-	0.2	0.2	0.2	-
Photoactivated bleach (ppm)	15	27	-	-	20	20
Photoactivated bleach 1 (ppm)	15	-	-	-	-	-
Brightener 1	0.08	0.2	-	-	0.09	0.15
Brightener 2	-	0.04	-	-	-	-
Perfume	0.3	0.5	0.4	0.3	0.4	0.3
Silicone anti- foam	0.5	2.4	0.3	0.5	0.3	2.0
Density in g/litre	750	750	750	750	750	750
Miscellaneous and minors	Up to 100%					

Example 6

The following detergent compositions of particular utility under European machine wash conditions were prepared according to the present invention :

5

	I	II	III	IV
Blown Powder				
LAS	6.0	5.0	11.0	6.0
TAS	2.0	-	-	2.0
Zeolite A	24.0	-	-	20.0
STPP	-	27.0	24.0	-
Sulfate	4.0	6.0	13.0	-
MA/AA	1.0	4.0	6.0	2.0
Silicate	1.0	7.0	3.0	3.0
CMC	1.0	1.0	0.5	0.6
Brightener 1	0.2	0.2	0.2	0.2
Silicone antifoam	1.0	1.0	1.0	0.3
DETPMP	0.4	0.4	0.2	0.4
Spray On				
Brightener	0.02	-	-	0.02
C45E7	-	-	-	5.0
C45E2	2.5	2.5	2.0	-
C45E3	2.6	2.5	2.0	-
Perfume	0.5	0.3	0.5	0.2
Silicone antifoam	0.3	0.3	0.3	-
Dry additives				
QEA	-	-	-	1.0
EDDS	0.3	-	-	-
Sulfate	2.0	3.0	5.0	10.0
Carbonate	6.0	13.0	15.0	14.0
Citric	2.5	-	-	2.0

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	I	II	III	IV
QAS 1	0.5	-	-	0.5
Na-SKS-6	10.0	-	-	-
Percarbonate	18.5	-	-	-
PB4	-	18.0	10.0	21.5
TAED	2.0	2.0	-	2.0
NACA-OBS	3.0	2.0	4.0	-
Protease	0.03	0.03	0.03	0.03
Mannanase	0.009	0.01	0.03	0.001
Lipase	0.008	0.008	0.008	0.004
Amylase	0.003	0.003	0.003	0.006
Brightener 1	0.05	-	-	0.05
Miscellaneous and minors			Up to 100%	

Example 7

The following granular detergent compositions were prepared according to the present invention :

	I	II	III	IV	V	VI
Blown Powder						
LAS	23.0	8.0	7.0	9.0	7.0	7.0
TAS	-	-	-	-	1.0	-
C45AS	6.0	6.0	5.0	8.0	-	-
C45AES	-	1.0	1.0	1.0	-	-
C45E35	-	-	-	-	2.0	4.0
Zeolite A	10.0	18.0	14.0	12.0	10.0	10.0
MA/AA	-	0.5	-	-	-	2.0
MA/AA 1	7.0	-	-	-	-	-
AA	-	3.0	3.0	2.0	3.0	3.0
Sulfate	5.0	6.3	14.3	11.0	15.0	19.3
Silicate	10.0	1.0	1.0	1.0	1.0	1.0
Carbonate	15.0	20.0	10.0	20.7	8.0	6.0
PEG 4000	0.4	1.5	1.5	1.0	1.0	1.0
DTPA	-	0.9	0.5	-	-	0.5
Brightener 2	0.3	0.2	0.3	-	0.1	0.3
Spray On						
C45E7	-	2.0	-	-	2.0	2.0
C25E9	3.0	-	-	-	-	-
C23E9	-	-	1.5	2.0	-	2.0
Perfume	0.3	0.3	0.3	2.0	0.3	0.3
Agglomerates						
C45AS	-	5.0	5.0	2.0	-	5.0
LAS	-	2.0	2.0	-	-	2.0
Zeolite A	-	7.5	7.5	8.0	-	7.5

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	I	II	III	IV	V	VI
Carbonate	-	4.0	4.0	5.0	-	4.0
PEG 4000	-	0.5	0.5	-	-	0.5
Misc (Water etc.)	-	2.0	2.0	2.0	-	2.0
Dry additives						
QAS	-	-	-	-	1.0	-
Citric	-	-	-	-	2.0	-
PB4	-	-	-	-	12.0	1.0
PB1	4.0	1.0	3.0	2.0	-	-
Percarbonate	-	-	-	-	2.0	10.0
Carbonate	-	5.3	1.8	-	4.0	4.0
NOBS	4.0	-	6.0	-	-	0.6
Methyl cellu- lose	0.2	-	-	-	-	-
Na-SKS-6	8.0	-	-	-	-	-
STS	-	-	2.0	-	1.0	-
Culmene sulfo- nic acid	-	1.0	-	-	-	2.0
Protease	0.02	0.02	0.02	0.01	0.02	0.02
Mannanase	0.009	0.01	0.03	0.009	0.01	0.001
Lipase	0.004	-	0.004	-	0.004	0.008
Amylase	0.003	-	0.002	-	0.003	-
Cellulase	0.0005	0.0005	0.000	0.000	0.000	0.000
			5	7	5	5
PVPVI	-	-	-	-	0.5	0.1
PVP	-	-	-	-	0.5	-
PVNO	-	-	0.5	0.3	-	-
QEA	-	-	-	-	1.0	-
SRP 1	0.2	0.5	0.3	-	0.2	-

	I	II	III	IV	V	VI
Silicone anti-foam	0.2	0.4	0.2	0.4	0.1	-
Mg sulfate	-	-	0.2	-	0.2	-
Miscellaneous and minors				Up to 100%		



Example 8

The following nil bleach-containing detergent compositions of particular use in the washing of colored clothing were prepared according to the present invention :

5

	I	II	III
Blown Powder			
Zeolite A	15.0	15.0	-
Sulfate	-	5.0	-
LAS	3.0	3.0	-
DETPMP	0.4	0.5	-
CMC	0.4	0.4	-
MA/AA	4.0	4.0	-
Agglomerates			
C45AS	-	-	11.0
LAS	6.0	5.0	-
TAS	3.0	2.0	-
Silicate	4.0	4.0	-
Zeolite A	10.0	15.0	13.0
CMC	-	-	0.5
MA/AA	-	-	2.0
Carbonate	9.0	7.0	7.0
Spray-on			
Perfume	0.3	0.3	0.5
C45E7	4.0	4.0	4.0
C25E3	2.0	2.0	2.0
Dry additives			
MA/AA	-	-	3.0
Na-SKS-6	-	-	12.0
Citrate	10.0	-	8.0
Bicarbonate	7.0	3.0	5.0

	I	II	III
Carbonate	8.0	5.0	7.0
PVPVI/PVNO	0.5	0.5	0.5
Protease	0.03	0.02	0.05
Mannanase	0.001	0.004	0.03
Lipase	0.008	0.008	0.008
Amylase	0.01	0.01	0.01
Cellulase	0.001	0.001	0.001
Silicone antifoam	5.0	5.0	5.0
Sulfate	-	9.0	-
Density (g/litre)	700	700	700
Miscellaneous and minors			Up to 100%

Example 9

The following detergent compositions were prepared according to the present invention :

	I	II	III	IV
Base granule				
Zeolite A	30.0	22.0	24.0	10.0
Sulfate	10.0	5.0	10.0	7.0
MA/AA	3.0	-	-	-
AA	-	1.6	2.0	-
MA/AA 1	-	12.0	-	6.0
LAS	14.0	10.0	9.0	20.0
C45AS	8.0	7.0	9.0	7.0
C45AES	-	1.0	1.0	-
Silicate	-	1.0	0.5	10.0
Soap	-	2.0	-	-
Brightener 1	0.2	0.2	0.2	0.2
Carbonate	6.0	9.0	10.0	10.0
PEG 4000	-	1.0	1.5	-
DTPA	-	0.4	-	-
Spray On				
C25E9	-	-	-	5.0
C45E7	1.0	1.0	-	-
C23E9	-	1.0	2.5	-
Perfume	0.2	0.3	0.3	-
Dry additives				
Carbonate	5.0	10.0	18.0	8.0
PVPVI/PVNO	0.5	-	0.3	-
Protease	0.03	0.03	0.03	0.02
Mannanase	0.002	0.009	0.015	0.03
Lipase	0.008	-	-	0.008

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	I	II	III	IV
Amylase	0.002	-	-	0.002
Cellulase	0.0002	0.0005	0.0005	0.0002
NOBS	-	4.0	-	4.5
PB1	1.0	5.0	1.5	6.0
Sulfate	4.0	5.0	-	5.0
SRP 1	-	0.4	-	-
Suds suppressor	-	0.5	0.5	-
Miscellaneous and minors			Up to 100%	

Example 10

The following granular detergent compositions were prepared according to the present invention :

	I	II	III
Blown Powder			
Zeolite A	20.0	-	15.0
STPP	-	20.0	-
Sulfate	-	-	5.0
Carbonate	-	-	5.0
TAS	-	-	1.0
LAS	6.0	6.0	6.0
C68AS	2.0	2.0	-
Silicate	3.0	8.0	-
MA/AA	4.0	2.0	2.0
CMC	0.6	0.6	0.2
Brightener 1	0.2	0.2	0.1
DETPMP	0.4	0.4	0.1
STS	-	-	1.0
Spray On			
C45E7	5.0	5.0	4.0
Silicone antifoam	0.3	0.3	0.1
Perfume	0.2	0.2	0.3
Dry additives			
QEA	-	-	1.0
Carbonate	14.0	9.0	10.0
PB1	1.5	2.0	-
PB4	18.5	13.0	13.0
TAED	2.0	2.0	2.0
QAS	-	-	1.0

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	I	II	III
Photoactivated bleach	15 ppm	15 ppm	15 ppm
Na-SKS-6	-	-	3.0
Protease	0.03	0.03	0.007
Mannanase	0.001	0.005	0.02
Lipase	0.004	0.004	0.004
Amylase	0.006	0.006	0.003
Cellulase	0.0002	0.0002	0.0005
Sulfate	10.0	20.0	5.0
Density (g/litre)	700	700	700
Miscellaneous and minors			Up to 100%

Example 11

The following detergent compositions were prepared according to the present invention :

	I	II	III
Blown Powder			
Zeolite A	15.0	15.0	15.0
Sulfate	-	5.0	-
LAS	3.0	3.0	3.0
QAS	-	1.5	1.5
DETPMP	0.4	0.2	0.4
EDDS	-	0.4	0.2
CMC	0.4	0.4	0.4
MA/AA	4.0	2.0	2.0
Agglomerate			
LAS	5.0	5.0	5.0
TAS	2.0	2.0	1.0
Silicate	3.0	3.0	4.0
Zeolite A	8.0	8.0	8.0
Carbonate	8.0	8.0	4.0
Spray On			
Perfume	0.3	0.3	0.3
C45E7	2.0	2.0	2.0
C25E3	2.0	-	-
Dry Additives			
Citrate	5.0	-	2.0
Bicarbonate	-	3.0	-
Carbonate	8.0	15.0	10.0
TAED	6.0	2.0	5.0
PB1	14.0	7.0	10.0
PEO	-	-	0.2

	I	II	III
Bentonite clay	-	-	10.0
Protease	0.03	0.03	0.03
Mannanase	0.001	0.005	0.01
Lipase	0.008	0.008	0.008
Cellulase	0.001	0.001	0.001
Amylase	0.01	0.01	0.01
Silicone antifoam	5.0	5.0	5.0
Sulfate	-	3.0	-
Density (g/litre)	850	850	850
Miscellaneous and minors			Up to 100%



Example 12

The following detergent compositions were prepared according to the present invention :

	I	II	III	IV
LAS	18.0	14.0	24.0	20.0
QAS	0.7	1.0	-	0.7
TFAA	-	1.0	-	-
C23E56.5	-	-	1.0	-
C45E7	-	1.0	-	-
C45E3S	1.0	2.5	1.0	-
STPP	32.0	18.0	30.0	22.0
Silicate	9.0	5.0	9.0	8.0
Carbonate	11.0	7.5	10.0	5.0
Bicarbonate	-	7.5	-	-
PB1	3.0	1.0	-	-
PB4	-	1.0	-	-
NOBS	2.0	1.0	-	-
DETPMP	-	1.0	-	-
DTPA	0.5	-	0.2	0.3
SRP 1	0.3	0.2	-	0.1
MA/AA	1.0	1.5	2.0	0.5
CMC	0.8	0.4	0.4	0.2
PEI	-	-	0.4	-
Sulfate	20.0	10.0	20.0	30.0
Mg sulfate	0.2	-	0.4	0.9
Mannanase	0.001	0.005	0.01	0.015
Protease	0.03	0.03	0.02	0.02
Amylase	0.008	0.007	-	0.004
Lipase	0.004	-	0.002	-
Cellulase	0.0003	-	-	0.0001

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	I	II	III	IV
Photoactivated bleach	30 ppm	20 ppm	-	10 ppm
Perfume	0.3	0.3	0.1	0.2
Brightener 1/2	0.05	0.02	0.08	0.1
Miscellaneous and minors			up to 100%	

Example 13

The following liquid detergent formulations were prepared according to the present invention (Levels are given in parts per weight, enzyme are expressed in pure enzyme) :

5

	I	II	III	IV	V
LAS	11.5	8.8	-	3.9	-
C25E2.5S	-	3.0	18.0	-	16.0
C45E2.25S	11.5	3.0	-	15.7	-
C23E9	-	2.7	1.8	2.0	1.0
C23E7	3.2	-	-	-	-
CFAA	-	-	5.2	-	3.1
TPKFA	1.6	-	2.0	0.5	2.0
Citric (50%)	6.5	1.2	2.5	4.4	2.5
Ca formate	0.1	0.06	0.1	-	-
Na formate	0.5	0.06	0.1	0.05	0.05
SCS	4.0	1.0	3.0	1.2	-
Borate	0.6	-	3.0	2.0	2.9
Na hydroxide	5.8	2.0	3.5	3.7	2.7
Ethanol	1.75	1.0	3.6	4.2	2.9
1,2 Propanediol	3.3	2.0	8.0	7.9	5.3
Monoethanolamine	3.0	1.5	1.3	2.5	0.8
TEPAE	1.6	-	1.3	1.2	1.2
Mannanase	0.001	0.01	0.015	0.015	0.001
Protease	0.03	0.01	0.03	0.02	0.02
Lipase	-	-	0.002	-	-
Amylase	-	-	-	0.002	-
Cellulase	-	-	0.0002	0.0005	0.0001
SRP 1	0.2	-	0.1	-	-
DTPA	-	-	0.3	-	-
PVNO	-	-	0.3	-	0.2

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	I	II	III	IV	V
Brightener 1	0.2	0.07	0.1	-	-
Silicone antifoam	0.04	0.02	0.1	0.1	0.1
Miscellaneous and water					

Example 14

The following liquid detergent formulations were prepared according to the present invention (Levels are given in parts per weight, enzyme are expressed in pure enzyme) :

5

	I	II	III	IV
LAS	10.0	13.0	9.0	-
C25AS	4.0	1.0	2.0	10.0
C25E3S	1.0	-	-	3.0
C25E7	6.0	8.0	13.0	2.5
TFAA	-	-	-	4.5
APA	-	1.4	-	-
TPKFA	2.0	-	13.0	7.0
Citric	2.0	3.0	1.0	1.5
Dodecenyl / tetradecenyl succinic acid	12.0	10.0	-	-
Rapeseed fatty acid	4.0	2.0	1.0	-
Ethanol	4.0	4.0	7.0	2.0
1,2 Propanediol	4.0	4.0	2.0	7.0
Monoethanolamine	-	-	-	5.0
Triethanolamine	-	-	8.0	-
TEPAE	0.5	-	0.5	0.2
DETPMP	1.0	1.0	0.5	1.0
Mannanase	0.001	0.015	0.01	0.03
Protease	0.02	0.02	0.01	0.008
Lipase	-	0.002	-	0.002
Amylase	0.004	0.004	0.01	0.008
Cellulase	-	-	-	0.002
SRP 2	0.3	-	0.3	0.1
Boric acid	0.1	0.2	1.0	2.0
Ca chloride	-	0.02	-	0.01

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	I .	II	III	IV
Brightener 1	-	0.4	-	-
Suds suppressor	0.1	0.3	-	0.1
Opacifier	0.5	0.4	-	0.3
NaOH up to pH	8.0	8.0	7.6	7.7
Miscellaneous and water				

Example 15

The following liquid detergent compositions were prepared according to the present invention (Levels are given in parts per weight, enzyme are expressed in pure enzyme) :

5

	I	II	III	IV
LAS	25.0	-	-	-
C25AS	-	13.0	18.0	15.0
C25E3S	-	2.0	2.0	4.0
C25E7	-	-	4.0	4.0
TFAA	-	6.0	8.0	8.0
APA	3.0	1.0	2.0	-
TPKFA	-	15.0	11.0	11.0
Citric	1.0	1.0	1.0	1.0
Dodecenyl / tetradecenyl succinic acid	15.0	-	-	-
Rapeseed fatty acid	1.0	-	3.5	-
Ethanol	7.0	2.0	3.0	2.0
1,2 Propanediol	6.0	8.0	10.0	13.0
Monoethanolamine	-	-	9.0	9.0
TEPAE	-	-	0.4	0.3
DETPMP	2.0	1.2	1.0	-
Mannanase	0.001	0.0015	0.01	0.01
Protease	0.05	0.02	0.01	0.02
Lipase	-	-	0.003	0.003
Amylase	0.004	0.01	0.01	0.01
Cellulase	-	-	0.004	0.003
SRP 2	-	-	0.2	0.1
Boric acid	1.0	1.5	2.5	2.5
Bentonite clay	4.0	4.0	-	-
Brightener 1	0.1	0.2	0.3	-

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	I	II	III	IV
Suds suppressor	0.4	-	-	-
Opacifier	0.8	0.7	-	-
NaOH up to pH	8.0	7.5	8.0	8.2
Miscellaneous and water				



Example 16

The following liquid detergent compositions were prepared according to the present invention (Levels are given in parts by weight, enzyme are expressed in pure enzyme) :

5

	I	II
LAS	27.6	18.9
C45AS	13.8	5.9
C13E8	3.0	3.1
Oleic acid	3.4	2.5
Citric	5.4	5.4
Na hydroxide	0.4	3.6
Ca Formate	0.2	0.1
Na Formate	-	0.5
Ethanol	7.0	-
Monoethanolamine	16.5	8.0
1,2 propanediol	5.9	5.5
Xylene sulfonic acid	-	2.4
TEPAE	1.5	0.8
Protease	0.05	0.02
Mannanase	0.001	0.01
PEG	-	0.7
Brightener 2	0.4	0.1
Perfume	0.5	0.3
Water and Minors		

Example 17

The following granular fabric detergent compositions which provide "softening through the wash" capability were prepared according to the present invention :

5

	I	II
C45AS	-	10.0
LAS	7.6	-
C68AS	1.3	-
C45E7	4.0	-
C25E3	-	5.0
Coco-alkyl-dimethyl hydroxy-ethyl ammonium chloride	1.4	1.0
Citrate	5.0	3.0
Na-SKS-6	-	11.0
Zeolite A	15.0	15.0
MA/AA	4.0	4.0
DETPMP	0.4	0.4
PB1	15.0	-
Percarbonate	-	15.0
TAED	5.0	5.0
Smectite clay	10.0	10.0
HMWPEO	-	0.1
Mannanase	0.001	0.01
Protease	0.02	0.01
Lipase	0.02	0.01
Amylase	0.03	0.005
Cellulase	0.001	-
Silicate	3.0	5.0
Carbonate	10.0	10.0
Suds suppressor	1.0	4.0

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CMC

I

II

0.2

0.1

Miscellaneous and minors

Up to 100%

Example 18

The following rinse added fabric softener composition was prepared according to the present invention :

DEQA (2)	20.0
Mannanase	0.0008
Cellulase	0.001
HCL	0.03
Antifoam agent	0.01
Blue dye	25ppm
CaCl <sub>2</sub>	0.20
Perfume	0.90
5 Miscellaneous and water	Up to 100%

Example 19

The following fabric softener and dryer added fabric conditioner compositions were prepared according to the present invention :

5

	I	II	III	IV	V
DEQA	2.6	19.0	-	-	-
DEQA (2)	-	-	-	-	51.8
DTMAMS	-	-	-	26.0	-
SDASA	-	-	70.0	42.0	40.2
Stearic acid of IV=0	0.3	-	-	-	-
Neodol 45-13	-	-	13.0	-	-
Hydrochloride acid	0.02	0.02	-	-	-
Ethanol	-	-	1.0	-	-
Mannanase	0.0008	0.0002	0.0005	0.005	0.0002
Perfume	1.0	1.0	0.75	1.0	1.5
Glycoperse S-20	-	-	-	-	15.4
Glycerol	-	-	-	26.0	-
monostearate					
Digeranyl Succinate	-	-	0.38	-	-
Silicone antifoam	0.01	0.01	-	-	-
Electrolyte	-	0.1	-	-	-
Clay	-	-	-	3.0	-
Dye	10ppm	25ppm	0.01	-	-
Water and minors	100%	100%	-	-	-

Example 20

The following laundry bar detergent compositions were prepared according to the present invention (Levels are given in parts per weight, enzyme are expressed in pure enzyme) :

5

	I	II	III	VI	V	III	VI	V
LAS	-	-	19.0	15.0	21.0	6.75	8.8	-
C28AS	30.0	13.5	-	-	-	15.7	11.2	22.5
						5		
Na Laurate	2.5	9.0	-	-	-	-	-	-
Zeolite A	2.0	1.25	-	-	-	1.25	1.25	1.25
Carbonate	20.0	3.0	13.0	8.0	10.0	15.0	15.0	10.0
Ca Carbon- ate	27.5	39.0	35.0	-	-	40.0	-	40.0
Sulfate	5.0	5.0	3.0	5.0	3.0	-	-	5.0
TSPP	5.0	-	-	-	-	5.0	2.5	-
STPP	5.0	15.0	10.0	-	-	7.0	8.0	10.0
Bentonite clay	-	10.0	-	-	5.0	-	-	-
DETPMP	-	0.7	0.6	-	0.6	0.7	0.7	0.7
CMC	-	1.0	1.0	1.0	1.0	-	-	1.0
Talc	-	-	10.0	15.0	10.0	-	-	-
Silicate	-	-	4.0	5.0	3.0	-	-	-
PVNO	0.02	0.03	-	0.01	-	0.02	-	-
MA/AA	0.4	1.0	-	-	0.2	0.4	0.5	0.4
SRP 1	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Mannanase	0.00	0.00	0.01	0.01	0.01	0.00	0.05	0.01
	1	1			5	1		
Amylase	-	-	0.01	-	-	-	0.00	-

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	I	II	III	VI	V	III	VI	V
Protease	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	1	4	1	3	3	1	1	3
Lipase	-	0.00	-	0.00	-	-	-	-
		2		2				
Cellulase	-	.000	-	-	.000	.000	-	-
		3			3	2		
PEO	-	0.2	-	0.2	0.3	-	-	0.3
Perfume	1.0	0.5	0.3	0.2	0.4	-	-	0.4
Mg sulfate	-	-	3.0	3.0	3.0	-	-	-
Brightener	0.15	0.1	0.15	-	-	-	-	0.1
Photoactiva	-	15.0	15.0	15.0	15.0	-	-	15.0
ted bleach								
(ppm)								

Example 21

The following detergent additive compositions were prepared according to the present invention :

	I	II	III
LAS	-	5.0	5.0
STPP	30.0	-	20.0
Zeolite A	-	35.0	20.0
PB1	20.0	15.0	-
TAED	10.0	8.0	-
Mannanase	0.001	0.01	0.01
Protease	0.3	0.3	0.3
Amylase	-	0.06	0.06
5 Minors, water and miscellaneous			Up to 100%



Example 22

The following compact high density (0.96Kg/l) dishwashing detergent compositions were prepared according to the present invention :

5

	I	II	III	IV	V	VI	VII	VIII
STPP	-	-	54.3	51.4	51.4	-	-	50.9
Citrate	35.0	17.0	-	-	-	46.1	40.2	-
Carbonate	-	17.5	14.0	14.0	14.0	-	8.0	32.1
Bicarbonat e	-	-	-	-	-	25.4	-	-
Silicate	32.0	14.8	14.8	10.0	10.0	1.0	25.0	3.1
Metasilica te	-	2.5	-	9.0	9.0	-	-	-
PB1	1.9	9.7	7.8	7.8	7.8	-	-	-
PB4	8.6	-	-	-	-	-	-	-
Percarbona te	-	-	-	-	-	6.7	11.8	4.8
Nonionic	1.5	2.0	1.5	1.7	1.5	2.6	1.9	5.3
TAED	5.2	2.4	-	-	-	2.2	-	1.4
HEDP	-	1.0	-	-	-	-	-	-
DETPMP	-	0.6	-	-	-	-	-	-
MnTACN	-	-	-	-	-	-	0.00	-
							8	
PAAC	-	-	0.00	0.01	0.00	-	-	-
			8		7			
BzP	-	-	-	-	1.4	-	-	-
Paraffin	0.5	0.5	0.5	0.5	0.5	0.6	-	-
Mannanase	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	1	1	2	2	1	3	2	2

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	I	II	III	IV	V	VI	VII	VIII
Protease	0.07	0.07	0.02	0.05	0.04	0.02	0.05	0.06
	2	2	9	3	6	6	9	
Amylase	0.01	0.01	0.00	0.01	0.01	0.00	0.01	0.03
	2	2	6	2	3	9	7	
Lipase	-	0.00	-	0.00	-	-	-	-
		1		5				
BTA	0.3	0.3	0.3	0.3	0.3	-	0.3	0.3
MA/AA	-	-	-	-	-	-	4.2	-
480N	3.3	6.0	-	-	-	-	-	0.9
Perfume	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.1
Sulphate	7.0	20.0	5.0	2.2	0.8	12.0	4.6	-
pH	10.8	11.0	10.8	11.3	11.3	9.6	10.8	10.9
Miscellaneous and water						Up to 100%		

Example 23

The following granular dishwashing detergent compositions of bulk density 1.02Kg/L were prepared according to the present invention :

5

	I	II	III	IV	V	VI	VII	VII
STPP	30.0	30.0	33.0	34.2	29.6	31.1	26.6	17.6
Carbonate	30.5	30.5	31.0	30.0	23.0	39.4	4.2	45.0
Silicate	7.4	7.4	7.5	7.2	13.3	3.4	43.7	12.4
Metasilicat	-	-	4.5	5.1	-	-	-	-
e								
Percarbonat	-	-	-	-	-	4.0	-	-
e								
PB1	4.4	4.2	4.5	4.5	-	-	-	-
NADCC	-	-	-	-	2.0	-	1.6	1.0
Nonionic	1.2	1.0	0.7	0.8	1.9	0.7	0.6	0.3
TAED	1.0	-	-	-	-	0.8	-	-
PAAC	-	0.00	0.004	0.00	-	-	-	-
		4		4				
BzP	-	-	-	1.4	-	-	-	-
Paraffin	0.25	0.25	0.25	0.25	-	-	-	-
Mannanase	0.00	0.00	0.001	0.00	0.00	0.00	0.00	0.00
	1	1		1	1	1	1	1
Protease	0.03	0.01	0.03	0.02	-	0.03	-	-
	6	5		8				
Amylase	0.00	0.00	0.01	0.00	-	0.01	-	-
	3	3		6				
Lipase	0.00	-	0.001	-	-	-	-	-
	5							
BTA	0.15	0.15	0.15	0.15	-	-	-	-
Perfume	0.2	0.2	0.2	0.2	0.1	0.2	0.2	-



Example 24

The following tablet detergent compositions were prepared according to the present invention by compression of a granular dishwashing detergent composition at a pressure of 13KN/cm<sup>2</sup> using a standard 12 head rotary press:

	I	II	III	IV	V	VI
STPP	-	48.8	49.2	38.0	-	46.8
Citrate	26.4	-	-	-	31.1	-
Carbonate	-	5.0	14.0	15.4	14.4	23.0
Silicate	26.4	14.8	15.0	12.6	17.7	2.4
Mannanase	0.001	0.001	0.001	0.001	0.001	0.02
Protease	0.058	0.072	0.041	0.033	0.052	0.013
Amylase	0.01	0.03	0.012	0.007	0.016	0.002
Lipase	0.005	-	-	-	-	-
PB1	1.6	7.7	12.2	10.6	15.7	-
PB4	6.9	-	-	-	-	14.4
Nonionic	1.5	2.0	1.5	1.65	0.8	6.3
PAAC	-	-	0.02	0.009	-	-
MnTACN	-	-	-	-	0.007	-
TAED	4.3	2.5	-	-	1.3	1.8
HEDP	0.7	-	-	0.7	-	0.4
DETPMP	0.65	-	-	-	-	-
Paraffin	0.4	0.5	0.5	0.55	-	-
BTA	0.2	0.3	0.3	0.3	-	-
PA30	3.2	-	-	-	-	-
MA/AA	-	-	-	-	4.5	0.55
Perfume	-	-	0.05	0.05	0.2	0.2
Sulphate	24.0	13.0	2.3	-	10.7	3.4
Weight of tablet	25g	25g	20g	30g	18g	20g

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	I	II	III	IV	V	VI
pH	10.6	10.6	10.7	10.7	10.9	11.2
Miscellaneous and water				Up to 100%		

Example 25

The following liquid dishwashing detergent compositions of density 1.40Kg/L were prepared according to the present invention :

5

	I	II	III	IV
STPP	17.5	17.5	17.2	16.0
Carbonate	2.0	-	2.4	-
Silicate	5.3	6.1	14.6	15.7
NaOCl	1.15	1.15	1.15	1.25
Polygen/carbopol	1.1	1.0	1.1	1.25
Nonionic	-	-	0.1	-
NaBz	0.75	0.75	-	-
Mannanase	0.001	0.005	0.01	0.001
NaOH	-	1.9	-	3.5
KOH	2.8	3.5	3.0	-
pH	11.0	11.7	10.9	11.0
Sulphate, miscellaneous and water	up to 100%			

Example 26

The following liquid dishwashing compositions were prepared according to the present invention :

	I	II	III	IV	V
C17ES	28.5	27.4	19.2	34.1	34.1
Amine oxide	2.6	5.0	2.0	3.0	3.0
C12 glucose amide	-	-	6.0	-	-
Betaine	0.9	-	-	2.0	2.0
Xylene sulfonate	2.0	4.0	-	2.0	-
Neodol C11E9	-	-	5.0	-	-
Polyhydroxy fatty acid amide	-	-	-	6.5	6.5
Sodium diethylene penta acetate (40%)	-	-	0.03	-	-
TAED	-	-	-	0.06	0.06
Sucrose	-	-	-	1.5	1.5
Ethanol	4.0	5.5	5.5	9.1	9.1
Alkyl diphenyl oxide disulfonate	-	-	-	-	2.3
Ca formate	-	-	-	0.5	1.1
Ammonium citrate	0.06	0.1	-	-	-
Na chloride	-	1.0	-	-	-
Mg chloride	3.3	-	0.7	-	-
Ca chloride	-	-	0.4	-	-
Na sulfate	-	-	0.06	-	-
Mg sulfate	0.08	-	-	-	-
Mg hydroxide	-	-	-	2.2	2.2
Na hydroxide	-	-	-	1.1	1.1
Hydrogen peroxide	200ppm	0.16	0.006	-	-



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	I	II	III	IV	V
Mannanase	0.001	0.05	0.001	0.00	0.01
				1	5
Protease	0.017	0.005	0.035	0.00	0.00
				3	2
Perfume	0.18	0.09	0.09	0.2	0.2
Water and minors				Up to 100%	

Example 27

The following liquid hard surface cleaning compositions were prepared according to the present invention :

	I	II	III	IV	V
Mannanase	0.001	0.0015	0.0015	0.05	0.01
Amylase	0.01	0.002	0.005	-	-
Protease	0.05	0.01	0.02	-	-
Hydrogen peroxide	-	-	-	6.0	6.8
Acetyl triethyl citrate	-	-	-	2.5	-
DTPA	-	-	-	0.2	-
Butyl hydroxy toluene	-	-	-	0.05	-
EDTA*	0.05	0.05	0.05	-	-
Citric / Citrate	2.9	2.9	2.9	1.0	-
LAS	0.5	0.5	0.5	-	-
C12 AS	0.5	0.5	0.5	-	-
C10AS	-	-	-	-	1.7
C12 (E) S	0.5	0.5	0.5	-	-
C12,13 E6.5 non- ionic	7.0	7.0	7.0	-	-
Neodol 23-6.5	-	-	-	12.0	-
Dobanol 23-3	-	-	-	-	1.5
Dobanol 91-10	-	-	-	-	1.6
C25AE1.8S	-	-	-	6.0	-
Na paraffin sul- phonate	-	-	-	6.0	-
Perfume	1.0	1.0	1.0	0.5	0.2
Propanediol	-	-	-	1.5	-

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	I	II	III	IV	V
Ethoxylated tetra- ethylene pen- tamine	-	-	-	1.0	-
2, Butyl octanol	-	-	-	-	0.5
Hexyl carbitol**	1.0	1.0	1.0	-	-
SCS	1.3	1.3	1.3	-	-
pH adjusted to	7-12	7-12	7-12	4	-
Miscellaneous and water				Up to 100%	
*Na4 ethylenediamine diacetic acid					
**Diethylene glycol monohexyl ether					

Example 28

The following spray composition for cleaning of hard surfaces and removing household mildew was prepared according to the present invention :

5

Mannanase	0.01
Amylase	0.01
Protease	0.01
Na octyl sulfate	2.0
Na dodecyl sulfate	4.0
Na hydroxide	0.8
Silicate	0.04
Butyl carbitol*	4.0
Perfume	0.35
Water/minors	up to 100%

\*Diethylene glycol monobutyl ether

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Lever, M. (1972) A new reaction for colormetric determination of carbohydrates. Anal. Biochem. 47, 273-279.

5

N. C. Carpita and D. M. Gibeaut (1993) The Plant Journal 3:1-30.

Diderichsen, B., Wedsted, U., Hedegaard, L., Jensen, B. R.,  
Sjøholm, C. (1990) Cloning of aldB, which encodes alpha-  
10 acetolactate decarboxylase, an exoenzyme from *Bacillus brevis*.  
J. Bacteriol. 172:4315-4321.

## SEQUENCE LISTING

Organism: *Bacillus halodurans*

Enzyme: Mannanase

5

SEQ ID NO:1

SEQUENCE CHARACTERISTICS:

LENGTH: 1051 base pairs

TYPE: nucleic acid

10 STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: genomic DNA

ORIGINAL SOURCE

15

FEATURE:

NAME/KEY: CDS

LOCATION: 1-1051

20 SEQUENCE DESCRIPTION: SEQ ID NO: 1

CTGGAAGGCG TTCAAATAAA TACGTTAATA GGAGTGGTTT TGTGTATAAG  
CTTACCCATA CGTATTTTGT TGCCTTAATT TGTTCTATTT TGATCTTTGC  
TGGGGTTTTTA AATACTTCTT CTTCAACAAGC AGAAGCCCAT CACAGTGGGT  
25 TCCATGTTAA TGGTACAACA TTATATGATG CAAATGGAAA CCCTTTTGTT  
ATGAGAGGGA TTAATCATGG ACATGCTTGG TTAAACAAG AACTAGAAAC  
ATCCATGAGA GGGATTAGTC AAACAGGGGC AAATACGATT CGTGTCGTTT  
TGTCTAATGG GCAAAGATGG CAAAAGATG ATCGAAACAT GGTAGCTTCG  
GTTATTTCTT TGGCAGAGCA GCATCAAATG ATTGCCGTTT TAGAAGTTCA  
30 TGATGCTACT GGTAGCAATA ATTTCTCCGA TCTGCAAGCT GCTGTGGACT  
ATTGGATTGA GATGAAGGAT GTTTTGCAGG GGAAAGAGGA CATAGTGATC  
ATTAATATCG CCAATGAATG GTACGGTGCT TGGGACGGAG GCGCATGGGC  
ACGAGGGTAT CAGAATGCGA TACGTCAGCT TCGAAATGCA GGCTTGTCAC  
ATACATTTAT GGTGACGCT GCCGGTTATG GCCAGTACCC TCAATCGGTA  
35 GTTGATTATG GTCAAGAAGT ATTAAATGCT GACCCACAGA GAAACACAAT  
GTTTTCTGTT CATATGTATG AATATGCAGG CGGAGATGCT AATACAGTAA  
GACGAAACAT TGA CTCGATC TTAAGCCAGA ACTTAGCTCT TGTCATTGGT  
GAATTCGGGC ATTGGCATTG TGACGGTGAT GTTGATGAGG ACACCATTTT

AAGCTATTCA CAGCAAAGAA ATGTGGGATG GTTGGCGTGG AGCTGGCATG  
GCAATAGTGA AGGGGTCGAA TATCTTGATT TATCGAATGA CTTTGCTGGT  
AATCGACTGA CATGGTGGGG TGATCGAATA GTAAACGGTC CGAATGGGAT  
TCGTCAAACC TCTAAAAGAA GCAGTGTGTT TCAATAAAGA AGTAAAATGA

5 A

SEQ ID NO:2

10

SEQUENCE CHARACTERISTICS:

LENGTH: 331 amino acids

TYPE: amino acid

TOPOLOGY: linear

15

MOLECULE TYPE: protein

SEQUENCE DESCRIPTION: SEQ ID NO: 2

VYKLTHTYFV ALICSILIFA GVLNTSSSQE EAHHS GFHVN GTTLYDANGN  
20 PFVMRGINHG HAWFKQELET SMRGISQTGA NTIRVVLSNG QRWQKDDRNM  
VASVISLAEQ HQMIAVLEVH DATGSNNFSD LQAAVDYWIE MKDVLQ GKED  
IVIINIANEW YGAWDGGAWA RGYQNAIRQL RNAGLSHTFM VDAAGYGQYP  
QSVVDYGQEV LNADPQRNTM FSVHMYEYAG GDANTVRRNI DSILSQNLAL  
VIGFEFGHWHY DGDVDEDTIL SYSQQRNVGW LAWSWHGNSE GVEYLDLSND  
25 FAGNRLTWWG DRIVNGPNGI RQTSKRSSVF Q

## CLAIMS

1. A mannanase which is

(a) a polypeptide produced by *Bacillus halodurans*, or

5 (b) a polypeptide comprising an amino acid sequence as shown in positions 33-331 of SEQ ID NO:2, or

(c) an analogue of the polypeptide defined in (a) or (b) which is at least 65% homologous with said polypeptide, or is derived from said polypeptide by substitution, deletion or addition of

10 one or several amino acids, or is immunologically reactive with a polyclonal antibody raised against said polypeptide in purified form.

2. An isolated polynucleotide molecule encoding a polypeptide

15 having mannanase activity selected from the group consisting of:

(a) polynucleotide molecules comprising a nucleotide sequence as shown in SEQ ID NO:1 from nucleotide 138 to nucleotide 1034;

(b) polynucleotide molecules that encode a polypeptide that is at least 65% identical to the amino acid sequence of SEQ ID NO:2  
20 from amino acid residue 33 to amino acid residue 331; and

(c) degenerate nucleotide sequences of (a) or (b).

3. The isolated polynucleotide molecule according to claim 2, wherein the polynucleotide is DNA.

25

4. An isolated polynucleotide molecule encoding a polypeptide having mannanase activity which polynucleotide molecule hybridizes to a denatured double-stranded DNA probe under medium

stringency conditions, wherein the probe is selected from the  
30 group consisting of DNA probes comprising the sequence shown in positions 42-1051 of SEQ ID NO:1, the sequence shown in positions 42-1034 of SEQ ID NO:1, the sequence shown in positions



138-1034 of SEQ ID NO:1 and DNA probes comprising a subsequence of positions 42-1051 of SEQ ID NO:1 having a length of at least about 100 base pairs.

- 5 5. An expression vector comprising the following operably linked elements: a transcription promoter; a DNA segment selected from the group consisting of (a) polynucleotide molecules encoding a polypeptide having mannanase activity comprising a nucleotide sequence as shown in SEQ ID NO:1 from nucleotide 138 to nucleotide 1034, (b) polynucleotide molecules encoding a polypeptide having mannanase activity that is at least 65% identical to the amino acid sequence of SEQ ID NO:2 from amino acid residue 33 to amino acid residue 331, and (c) degenerate nucleotide sequences of (a) or (b); and a transcription terminator.

15

6. A cultured cell into which has been introduced an expression vector according to claim 5, wherein said cell expresses the polypeptide encoded by the DNA segment.

- 20 7. An isolated polypeptide having mannanase activity selected from the group consisting of:  
(a) polypeptide molecules comprising an amino acid sequence as shown in SEQ ID NO: 2 from residue 33 to residue 331; and  
(b) polypeptide molecules that are at least 65% identical to the amino acids of SEQ ID NO: 2 from amino acid residue 33 to amino acid residue 331.

25

8. The polypeptide according to claim 7 which is produced by a strain belonging to the species *Bacillus halodurans*.

30

9. An enzyme preparation comprising a purified polypeptide according to claim 7.

10. A method of producing a polypeptide having mannanase activity comprising culturing a cell into which has been introduced an expression vector according to claim 5, whereby said cell  
5 expresses a polypeptide encoded by the DNA segment; and recovering the polypeptide.

11. The preparation according to claim 9 which further comprises one or more enzymes selected from the group consisting of prote-  
10 ases, cellulases (endoglucanases),  $\beta$ -glucanases, hemicellulases, lipases, peroxidases, laccases,  $\alpha$ -amylases, glucoamylases, cutinases, pectinases, reductases, oxidases, phenoloxidases, ligninases, pullulanases, pectate lyases, xyloglucanases, xylanases, pectin acetyl esterases, polygalacturonases, rhamnogalac-  
15 turonases, pectin lyases, other mannanases, pectin methylesterases, cellobiohydrolases, transglutaminases; or mixtures thereof.

12. An isolated enzyme having mannanase activity, in which the  
20 enzyme is (i) free from homologous impurities, and (ii) produced by the method according to claim 9.

13. A method for improving the properties of cellulosic or synthetic fibres, yarn, woven or non-woven fabric in which  
25 method the fibres, yarn or fabric is treated with an effective amount of the preparation according to claim 8 or an effective amount of the enzyme according to claim 1.

14. The method according to claim 13, wherein the enzyme preparation or the enzyme is used in a desizing process step.  
30

15. A method for degradation or modification of plant material in which method the plant material is treated with an effective amount of the preparation according to claim 8 or an effective amount of the enzyme according to claim 1.

5

16. The method according to claim 14 wherein the plant material is recycled waste paper; mechanical, chemical, semichemical, kraft or other paper-making pulps; fibres subjected to a retting process; or guar gum or locust bean gum containing material.

10

17. A method for processing liquid coffee extract, in which method the coffee extract is treated with an effective amount of the preparation according to claim 8 or an effective amount of the enzyme according to claim 1.

15

18. A cleaning composition comprising the enzyme preparation according to claim 8 or the enzyme according to claim 1.

19. The cleaning composition according to claim 18 which further  
20 comprises an enzyme selected from cellulases, amylases, pectin degrading enzymes and/or xyloglucanases; and another detergent ingredient.

20. The cleaning composition according to claim 18 wherein said  
25 enzyme or enzyme preparation is present at a level of from 0.0001% to 2%, preferably from 0.0005% to 0.5%, more preferably from 0.001% to 0.1% pure enzyme by weight of total composition.

21. The cleaning composition according to claim 19 wherein the  
30 enzyme is present at a level of from 0.0001% to 2%, preferably from 0.0005% to 0.5%, more preferably from 0.001% to 0.1% pure enzyme by weight of total composition.

22. The cleaning composition according to claim 19 wherein the enzyme is an amylase.

5 23. The cleaning composition according to claim 22 which further comprises yet another enzyme selected from cellulase, pectin degrading enzyme and/or xyloglucanase.

24. The cleaning composition according to claim 19 wherein the  
10 further enzyme is alkaline.

25. The cleaning composition according to claim 19 which further comprises a surfactant selected from anionic, nonionic, cationic surfactant, and/or mixtures thereof.

15

26. The cleaning composition according to claim 19 which further comprises a bleaching agent.

27. The cleaning composition according to claim 19 which further  
20 comprises a builder.

28. A fabric softening composition according to claim 19 which further comprises a cationic surfactant comprising two long chain lengths.

25

29. A process for machine treatment of fabrics which process comprises treating fabric during a washing cycle of a machine washing process with a washing solution containing the enzyme preparation according to claim 8 or the enzyme according to  
30 claim 1.

30. Use of the enzyme preparation according to claim 8 or the enzyme according to claim 1 together with a enzyme selected from cellulase, amylase, pectin degrading enzyme and/or xyloglucanase in a cleaning composition for fabric cleaning and/or fabric  
5 stain removal.

31. Use of the enzyme preparation according to claim 8 or the enzyme according to claim 1 together with a enzyme selected from cellulase, amylase, pectin degrading enzyme and/or xyloglucanase  
10 in a cleaning composition for cleaning hard surfaces such as floors, walls, bathroom tile and the like.

32. Use of the enzyme preparation according to claim 8 or the enzyme according to claim 1 together with a enzyme selected from  
15 cellulase, amylase, pectin degrading enzyme and/or xyloglucanase in a cleaning composition for hand or machine dishwashing.

33. Use of the enzyme preparation according to claim 8 or the enzyme according to claim 1 together with a enzyme selected from  
20 cellulase, amylase, pectin degrading enzyme and/or xyloglucanase in a cleaning composition for oral, dental, contact lenses and personal cleaning applications.

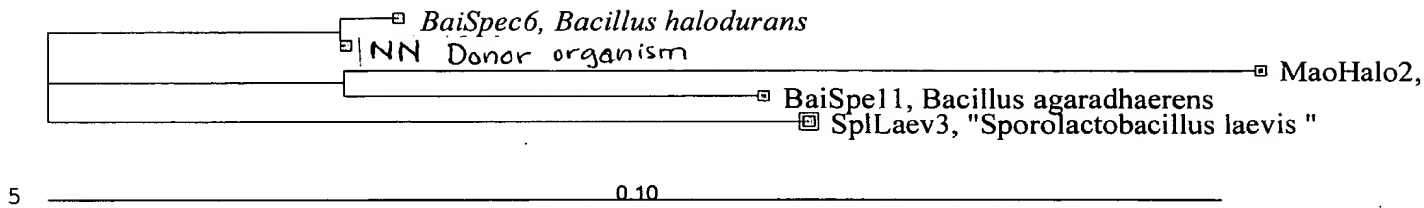


FIGURE 1

## ABSTRACT

## 5 NOVEL MANNANASES

Novel mannanases comprising an amino acid sequence as shown in positions 33-331 of SEQ ID NO:2 or their homologues may be derived from eg *Bacillus halodurans*, or may be encoded by  
10 polynucleotide molecules comprising a nucleotide sequence as shown in SEQ ID NO: 1 from nucleotide 138 to nucleotide 1034, polynucleotide molecules that encode a polypeptide that is at least 65% identical to the amino acid sequence of SEQ ID NO: 2 from amino acid residue 33 to amino acid residue 331, or degen-  
15 erate nucleotide sequences thereof. The mannanases are alkaline and are useful e.g. in cleaning compositions, for modifying plant material, and for treatment of cellulosic fibres.